UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE March 24, 1981

SUBJECT Regional Lead on Desloge/Big River Site

FROM William W Rice

RECEIVED

MAR 25 1981

ENFORCEMENT DIVISION

TO David Wagoner
Louise Jacobs
Paul Walker
John Morse
Mike Cole

I understand that the Missouri Department of Natural Resources is currently negotiating with St Joe Minerals in an attempt to develop a solution to the environmental problems posed by the Desloge site The region has indicated to the State that we wish to be kept informed about the status of their negotiations, however, I believe that it is inappropriate for our agency to take any action (such as federal enforcement, use of superfund, etc), unless the State is unable to reach a reasonable agreement that will provide a long-term solution to the problem I have therefore asked the Air and Hazardous Materials Division to take the lead on this activity until we decide that some other actions are warranted I will expect ARHM to maintain close contact with the State during this period and to keep me and other divisions--particularly Enforcement and S&A--informed about the status of the State's negotiations These divisions should also be given the opportunity to review Missouri's agreement with St Joe before we develop our final regional position on this issue

I have been impressed with the effort that Bill Ward in particular has put into this project I am requesting Enforcement to continue to make him available for assistance and I am encouraging ARHM to take advantage of the work he has already done

Please let me know if you have any questions or comments

cc C Hajinian
B Ward
B Keffer

m 1320 6 (R 3 76)

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SUPERFUND RECORDS

December 14, 1981

St Joe Mineral's Agreement with MDNR on Liability and Remedial Action for Lead Mine Tailings Site at Desloge, Missouri

Karen Flournoy, HAZM/TSS

Robert L. Morby, Chief, HAZM

THRU Katie Biggs, HAZM/TSS-CR&A
Dennis Degner, HAZM/TSS-PMTS

This memo summarizes developments with the abandoned lead mine tailings site at Desloge, Missouri and reviews the agreement between St Joe Minerals Corporation, Missouri Department of Natural Resources and other agencies to clean-up/stabilize the site The initial review was by Paul Doherty with additional review by myself

I Background

Lead mine tailings were deposited at the Desloge, Missouri site by St Joe Minerals Corporation for 29 years, between 1929 and 1958 The site covers approximately 500 acres The tailings are reportedly 2 - 4 percent lead and are piled to depths of up to 100 feet inside a horseshoe bend of the Big River

In 1972, the property was donated by St Joe Minerals to St Francois County The County in turn donated the land to the St Francois County Environmental Corporation, a non-profit organization for the purpose of establishing a sanitary landfill on the site Up to this time it is reported that the tailings site had been adequately maintained with no apparent incidents of tailing pile washout or erosion into the Big River

In 1977, a major washout occurred, reportedly as a result of a blocked drainage structure and neglected maintenance It is estimated that up to 50,000 cubic yards of lead tailings washed into the Big River. Minor erosion has continued up to the present time adding to the tailings deposited in the River

Following the washout incident, several studies were undertaken to assess the extent of environmental damage and explore remedial action alternatives

In late 1977, EPA/SVAN conducted an intensive survey of the Big River The general finding was that the Big River was degraded by mine tailings, mainly as a result of physical changes rather than toxicity It is reported that mine tailing deposits are the primary constituent of the stream bed for several miles downstream of the tailings pile

ARHM/HAZM-TSS PDoherty KFlournoy lmh x6531 10-16-81 Rev12-14-81 Disk H

TSS	TSS	TSS	HAZM
Flournoy	Biggs	Degner	Morby

In June 1979, a study was initiated by the University of Missouri to evaluate the present and potential problems of the site and to propose solutions to these problems Their report was issued in January 1980

A July 18, 1980, study by the Missouri Department of Conservation reported elevated levels of lead in the flesh of sucker fish downstream from the tailings pile As a result of these findings the Missouri Department of Health issued a warning to the public against eating bottom feeding fish in this area

Since the occurrence of the washout incident a number of meetings have been held among interested agencies to coordinate mitigation efforts Up until last year, enforcement action against St Joe Minerals was considered as the most likely course of action by several of these agencies The Corps of Engineers (COE) referred the case to the Department of Justice (DOJ) in late 1978 To date, no action has been taken by the DOJ on this referral In 1980, the recommendation from EPA/ENFC called for immediate enforcement action under Section 311 of the CWA and Section 7003 of RCRA The site was recommended for listing as an uncontrolled site by EPA, COE, and the Department of the Interior for potential Superfund action

In late 1980, MDNR began negotiating with St Joe Mineral Corporation for voluntary clean-up of the mine tailing site As a result of these negotiations, and the fact that mining waste became excluded from the hazardous waste regulation (November 19, 1980), the Regional Office agreed to suspend further enforcement actions until the results of these negotiations became known The Agency also agreed, at the request of MDNR, to drop the Desloge Lead Mine Tailings Site from the list of uncontrolled sites The negotiations continued from late 1980, until August 1981 It is understood that the principle stumbling blocks to negotiating the final agreement were

1 Reconciliation of St Joe Minerals' past and future liability for the site, and

2 Assigning responsibility for future site maintenance

The final negotiated agreement, "Covenant Not to Sue" between the St Joe Minerals Company, St Francois Environmental Corporation, State Department of Natural Resources, State Clean Water Commission, State Conservation Commission, and State Attorney General's Office was signed on September 4 1981

II The Agreement

The format of the Agreement, titled "Covenant Not to Sue" is in three basic parts These parts can be described as

1 Summary statements on the history of the site and washout incident

2 Statements of liability, responsibility and exemption from future litigation and

3 Description of remedial action work

The first several provisions of the Covenant set forth the history of the site and washout incident as described previously in this memo

The succeeding provisions set forth the conditions of liability, future litigation and remedial action responsibilities According to these provisions, St Joe Minerals and the St Francois County Environmental Corporation are released from responsibility for all damage, past and future, resulting from the washout incident The St Francois County Environmental Corporation assumes responsibility for contracting all agreed-upon remedial action work and assumes responsibility for future maintenance of the area The St Joe Minerals Corporation agrees to pay for the proposed work, provide supplemental fill material for reconstructing containment dams, and will provide "advisory" technical assistance to the St Francois County Environmental Corporation with the review and inspection of the construction work performed No responsibility for the work is assumed by St Joe Mineral with this "advisory" role

The third part of the Agreement provides specific details on the remedial action tasks This work is described in a document titled, "Repair of Damage at Desloge Landfill Along Big River," and has been made part of the Covenant by reference

The work can be briefly summarized as follows

1 Fill-in/repair of all major erosion gaps (two large gaps and three smaller gaps),

2 Reconstruction of three retaining berms at the repaired erosion areas,

3 Alteration of the failed drainage structure to prevent future blockage problems,

4 Seeding and fertilizer application to a 20 acre "demonstration" plot, and

5 Construction of all necessary haulage roads

III <u>Discussion</u>

Although the entire Repair of Damage at Desloge Landfill Along Big River document was not submitted to the Agency for review (drawings and photograph exhibits were omitted), the description of work is consistent with recommendations made in the 1980 report prepared by the University of Missouri for MDNR It appears that the agreed-upon work is a "middle ground" response to the University of Missouri report recommendations The major structural failures on the site will be remedied by the proposed work and this will eliminate much of the environmental hazards posed by future erosion or washouts at the site The University of Missouri performed extensive analyses on the engineering properties of the tailings material With certain exceptions (i e , areas where remedial work is planned) the report concluded that the tailing pile site "as a whole appears to be stable "

The proposed work does not address several existing or potential environmental hazards identified in the University of Missouri's report and other agency memoranda These potential environmental hazards are discussed briefly as follows

1 Contamination of Big River Benthic Zone and Fish Population Studies conducted by EPA and MDNR have documented that the Big River has been degraded for several miles downstream from the tailing site and that bottom feeding fish have elevated levels of lead in their flesh Both situations warrant concern from an environmental standpoint However, reclamation or dredging of the river is not included in the Agreement's scope of work Omitting this work from the Agreement appears justified. It would be unreasonable to expect St Joe Mineral to assume responsibility for a major dredging operation resulting from a washout incident which had occurred several years after the Company relinquished title (and responsibility) of the property to the County The State Department of Conservation and MDNR believe that a dredging operation would completely destroy the ecology of the river and that natural processes would be more effective in reclaiming the river given time

On the other hand, the Corps of Engineers and Department of Interior support a dredging operation and believe that the river bottom lead deposits pose a significant environmental hazard Both agencies initially favored listing the Big River Site as an uncontrolled site, eligible for Superfund action However, mining wastes are now excluded from Superfund Based on a review of the available information, the position of the MDNR appears to be a reasonable approach There have been no known reports of contaminated water supplies The local population has been discouraged from eating sucker fish caught in the area and as long as reasonable dietary precautions are taken health problems should not develop

2 Leachate Contamination (Heavy Metals) from the Sanitary Landfill The study conducted by the University of Missouri reported that liquid leachate from the landfill operation could lead to potentially serious contamination of water supplies Their laboratory studies, conducted with mine tailing material, showed that under acidic conditions, lead and zinc in the tailings can become soluble, migrate with leaching water flows and could eventually contaminate surface and groundwater supplies The report viewed the hazard of heavy metal leaching to be serious enough for MDNR to establish an "immediate monitoring program In March 1980, following the University of Missouri report, MDNR did perform one leachate monitoring at the sites Their results showed that the levels of lead, zinc and cadmium were not elevated above background levels nor did they exceed USPHS drinking water standards EMC/LCGL has questioned whether the samples analyzed by MDNR are representative of the Desloge landfill leachate We concur that the question of landfill leachate mobilizing heavy metals, particularly lead, has not been answered to date We recommend that a study be initiated to determine if the landfill leachate mobilizes heavy metals, in particular lead The Bureau of Mines is conducting a study on the lead tailings They should be contacted for background information and coordination for any additional studies in the area Alternately, two to three properly placed monitoring wells should be installed at the landfill with analysis to include lead and leachate parameters

The landfill situation does warrant the future attention o_ MDNR Periodic monitoring of leachate samples by MDNR should continue and appropriate actions taken if a problem develops MDNR and the State Conservation Commission have been accorded the right of access_for inspection purposes in the Agreement Monitoring and inspections are necessary

3 Site Stabilization, Revegetation and Hazards of Airborne Lead Dust The University of Missouri report concluded that the Desloge lead tailing pile will "remain a potential health hazard due to blowing of lead laden dust and the potential for further erosion until such time as the site is completely stabilized by vegetative growth " Because of problems with seed germination, moisture retention and fertilization, revegetation of the site will not occur through natural processes Although the Agreement provides for seeding and fertilizer application to 20 acres of land, this seeding operation will involve less than 5 percent of the tailing site It is understood that the seeded/fertilized plot may serve as a demonstration study to assess plant supporting characteristics of the tailing pile and that this study would provide the basis for future seeding and fertilization The Agreement is not specific on who, if anyone, is responsible for maintaining or evaluating the 20 acre demonstration plot

Revegetation of 20 acres still leaves over 95 percent of the Desloge site without plant cover Questions have been raised as to whether the potential for windblown lead dust at the abandoned lead tailing site represents a significant environmental hazard In the absence of specific air monitoring data, it is difficult to accurately assess the hazards posed by this exposure route A brief review of the available literature indicates that the environmental hazards associated with inhalation of lead and lead compounds during lead ore mining, crushing and milling operations is low The NIOSH development document for "Criteria for a Recommended Standard for Occupational Exposure to Inorganic Lead (1978)" provides a general overview of the degrees of occupational exposure to lead for 34 industrial operations Lead mining is not mentioned in this overview, suggesting that the hazards of occupational exposure may not be significant

The EPA publication, "Air Quality Criteria for Lead" (EPA 600/8-77-017) states

> Exposures for workers involved in lead mining depend to some extent on the solubility of the lead from the ores The lead sulfide (PbS) in galena is insoluble, and absorption through the lung may be slight It is not really known how readily absorption takes place In the stomach, however, some lead sulfide may be converted to slightly soluble lead chloride, which may then be absorbed in moderate amounts

Although occupational exposure to atmospheric lead is discussed in some detail in this publication, no further reference to lead mining exposure hazards is provided

Lead toxicity is mainly the result of the concentration of diffusable (soluble) lead in soft tissues of the body The insolubility of lead sulfide (galena) probably accounts for its low reported toxicity The "Registry of the Toxic Effects of Chemical Substances states that lead sulfide presents an "insignificant hazard" with regard to aquatic toxicity This is the lowest possible rating The low toxicity rating may also explain the lack of toxic reactions observed by EPA in the Big River following the washout incident

The Mine Safety and Health Administration (MSHA) is responsible for establishing and enforcing standards for occupational exposure to lead during mining operations The standard is 0 15 mg/m^3 of lead and lead compounds Mr Terry Phillips, Sub-district Manager of the MSHA Rolla, Missouri office states that compliance measurements for this standard are usually collected near the ore concentration operation Although this operation produces a concentrate which is 98 - 99 percent lead sulfide, compliance with the 0 15 mg/m^3 standard is not unusually difficult Given that the lead tailings are 2 - 4 percent lead, Mr Phillips did not believe that the abandoned tailing pile would violate their standard of 0 15 mg/m^3 Short-term violations may occur during periods of high winds but one would expect that due to the high density particulate nature of lead dust only the area immediately adjacent to and downwind from the site would be impacted Due to the low toxicity of lead sulfide, the low concentration of lead in the tailing pile and the intermittent nature of windblown occurrences, it is concluded that the environmental hazards posed by windblown tailing dust is not significant It may be advisable to establish ambient air quality monitoring stations near the site to confirm this conclusion

IV Summary

The Agreement (Covenant Not to Sue) between St Joe Minerals Corporation, DNR and interested agencies is a reasonable negotiated settlement to clean-up and remedy a tailing pile washout incident for which no party is clearly responsible The proposed remedial work will stabilize the site to prevent future washout problems but does not address other environmental concerns regarding

1 Tailings in the Big River sediment,

2 Potential leachate contamination from the landfill operation, and

3 The lack of a vegetative cover to further stabilize the site

4 Erosion control on a continuing basis

5 Long-term sampling/environmental evaluation program

Overall the Agreement addresses landfill dam repairs but did not include the above listed concerns as applicable The fact that lead concentrations in bottom feeding fish is high enough that the State issued a warning against their consumption is evidence to support a Federal action under §7003 of RCRA For this reason and because the above listed concerns are not addressed in the Agreement, EPA should continue to monitor the progress of the State It is recommended that we issue a letter to MDNR expressing our concerns and recommended actions they should undertake to address these

6 10

United States Department of the Interior
FISH AND WILDLIFE SERVICE
COLUMBIA NATIONAL FISHERIES RESEARCH LABORATORY
ROUTE 1
COLUMBIA MISSOURI 65201

IN REPLY REFER TO

April 25, 1985

William H Ward,
Assistant Regional Council
U S EPA, Region VII
324 E 11th St
Kansas City, MO 64106

Dear Mr Ward

Enclosed please find a copy of a Master's Thesis by John Besser which deals with the availability and effects of metals in leachates from Desloge mine tailings This study was part of a joint investigation in which the UMC Department of Forestry, Fisheries and Wildlife, The Environmental Trace Substances Research Center, and CNFRL participated Mr Besser is currently employed at our laboratory, please feel free to contact either of us should you care to discuss any aspect of this report

Sincerely,

Chris

Christopher J Schmitt

CJS efh

Enclosure

MAY 6 1985

KL 6/A

BIOAVAILABILITY AND TOXICITY OF HEAVY METALS IN MINE TAILINGS LEACHATE TO AQUATIC INVERTEBRATES

A Thesis

Presented to

the Faculty of the Graduate School

University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

John M. Besser

Charles F. Rabeni Thesis Supervisor

May 1985

The undersigned, appointed by the Dean of the Graduate Faculty, have examined a thesis entitled

Bioavailability and toxicity of heavy metals in mine tailings leachate to aquatic invertebrates

presented by

John M. Besser

a candidate for the degree of Master of Science

and hereby certify that in their opinion it is worthy of acceptance

Charles F. Rabeni

John R. Jones

[illegible]

ABSTRACT

Erosion and leaching of large deposits of lead mine tailings in the "Old Lead Belt" of southeast Missouri have led to extensive contamination of streams of the Big River drainage with toxic heavy metals Although revegetation of the tailings piles to reduce erosion has been proposed, the effects of revegetation on the release of metals from the tailings have not been studied In this study, aquatic invertebrates were exposed to leachates from test plots of tailings to evaluate the effects of cover materials on the bioavailability and toxicity of metals in tailings leachates

Bioaccumulation of metals from test plot leachates was increased in tailings plots with cover treatments of vegetation (seed/fertilizer and sod treatments) or organic matter (sludge and leaf treatments), relative to uncovered tailings or uncontaminated crushed dolomite Differences in metal bioaccumulation among treatments corresponded to dissolved metal concentrations in leachates, although invertebrates were apparently able to accumulate metals from ingested solids as well Formation of complexes with dissolved organic compounds led to high metal concentrations in leachate from the leaf treatment, which showed the highest metal bioaccumulation

Toxic effects of leachates on survivorship of crayfish and survivorship, growth and development of midge larvae showed similar trends among cover material treatments

Toxicity of leachates was more strongly correlated with water metal concentrations than with accumulated metals, suggesting that not all accumulated metals exerted toxic action Significant adverse effects on invertebrates occurred in this study at metal concentrations comparable to those measured in the Big River system and in seepage from tailings piles

The benefits of revegetation of the large tailings piles in the Old Lead Belt probably outweigh the adverse effects of cover materials on leachate formation However, the processes observed in this study probably also act on tailings already eroded into stream and riparian habitats, posing a long-term threat of metal toxicity to aquatic biota and human consumers of contaminated fish

ACKNOWLEDGEMENTS

I would like to express my thanks to the Columbia National Fisheries Research Laboratory of the U S Fish and Wildlife Service, for funding my research and providing access to the excellent facilities at CNFRL The staff of CNFRL deserves special thanks for their assistance throughout the study Chris Schmitt and Susan Finger provided invaluable advice and assistance with the study design as well as the dirty work of construction and maintenance of the study site Bill Brumbaugh helped me deal with the complexities of atomic absorption analysis Ed Henry and Dennis Chester provided midges and mayflies for leachate exposures as well as advice on invertebrate culture and bioassay techniques

I would like to thank my advisor, Dr Charles Rabeni, who provided both critical guidance and valued friendship throughout the study, while allowing me the freedom to make (and learn from) my own mistakes Thanks are also due to Drs John R Jones and S Roy Koirtyohann for their constructive criticism of this thesis Drs Terry R Finger and Mary G Henry graciously provided critical reviews of the project proposal

My fellow graduate students deserve special acknowledgement for the the many large and small ways they helped make my stay at UMC successful and rewarding Particularly helpful were the work and advice of Gary Whelan and Matt Knowlton, which provided much of the groundwork for this study John Harwood shared his insights into the arcane world of trace metal chemistry and provided the water chemistry data on which I relied so heavily in preparing this thesis

Finally, I would like to thank my parents for their unfailing support, both moral and financial, throughout my educational career Their faith in my abilities and encouragement of my career choices are greatly appreciated

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BACKGROUND

Southeast Missouri has long been a leading producer of lead in the United States Deposits were discovered in the early 1700's and surface mining was extensive by 1800 (Kramer 1976) These scattered mining activities eventually focused on an area of St Francois county, Missouri, which had extensive surface and subsurface deposits of the lead ore galena (PbS) By the mid-1800s, surface deposits were depleted and deep-shaft mining in the Bonneterre dolomite formation became predominant During the period 1907-1954 the area, known as the Old Lead Belt, was the largest producer of lead in the nation and all holdings in the area were acquired by the St Joseph Mining Company As these ore deposits became depleted and deposits of high grade ore were developed to the west (the New Lead Belt or Viburnum Trend), mines in the Old Lead Belt were closed between 1961 and 1972 (Kramer 1976)

Along with the 7 3 million metric tons of lead extracted between 1864 and 1972, mines in the Old Lead Belt generated some 227 million metric tons of tailings, by-products of separation of galena from the low grade lead ores (Kramer 1976) Milling processes used in the Old Lead Belt increased in sophistication during this period, from the early process of gravity fractionation or "jigging" to more recent flotation procedures which used a variety of reagents to assist in separating lead particles from ground ore As a result the tailings, deposited in large piles (up

LIST OF FIGURES

adjacent to the tailings deposits was heavily contaminated with tailings and that significant metal contamination of Big River water, sediment, fish and aquatic invertebrates extended at least 96 km downstream from the major tailings input at Desloge (Schmitt and Finger 1982, Whelan 1983) An engineering study of the Desloge tailings pile (Novak and Hasselwander 1980) determined that inadequate maintenance of drainage structures led to the failure of the tailings berm and the resultant massive erosion of tailings into the Big River The report presented guidelines for re-grading unstable slopes and modifying drainage patterns to reduce erosion problems, but concluded that seeding and addition of organic matter to establish vegetation cover would be required to permanently stabilize the tailings piles

Several researchers have emphasized the importance of biological and chemical processes which can mobilize heavy metals from tailings deposits Metals can be mobilized by weathering processes within the tailings piles (Kramer 1976) or by plants grown in tailings (Wixson et al 1983) Results of experimental leaching and revegetation studies (Novak and Hasselwander 1980) indicated that the solubility and toxicity of tailings metals could be increased by dissolved organic compounds, such as those generated by the landfill operations at Desloge, or from the application of organic mulches to aid revegetation Chemical and biological processes in aquatic systems can lead to increased dispersal and biological availability of heavy metals (Schmitt and Finger 1982, Whelan 1983) to 6 5 km^2) adjacent to milling sites, differ considerably in their content of lead, zinc and other heavy metals depending on the milling process used at the time of their deposition (Harwood 1984) As the tailings piles were abandoned or turned over to new ownership, adverse impacts of tailings on water quality and biota of nearby streams became evident Surveys conducted by the Missouri Department of Conservation attributed impacts on the aquatic invertebrate community of Flat River Creek to erosion of tailings piles near Elvins and Flat River, Missouri (Ryck 1974) A study of the impacts of mine tailings on Flat River Creek (Kramer 1976) found elevated levels of lead, zinc, cadmium and copper in water, sediments and biota of the stream and documented inputs of these metals to the stream from both erosion of tailings and inputs of seepage water from the Elvins tailings pile

Further attention was focused on environmental problems associated with the tailings deposits following major erosion events in 1977 which deposited about 38,000 cubic meters of tailings from the tailings pile at Desloge, Missouri into the channel of the Big River, the major stream draining the Old Lead Belt (Novak and Hasselwander 1980) Subsequent studies by the Missouri Department of Conservation found elevated levels of lead in fish collected from the Big River and local residents were cautioned against consumption of contaminated fish (Czarneski 1980, 1984) An extensive survey conducted by the U S Fish and Wildlife Service found that the reach of the Big River

STUDY SITE

Leachate Collection

Six experimental leaching plots were constructed on the grounds of the Columbia National Fisheries Research Laboratory (CNFRL), U S Fish and Wildlife Service, Columbia, Missouri Plots consisted of wooden frames (dimensions 3 7 m X 1 8 m X 0 3 m) lined with vinyl plastic A control plot was filled with uncontaminated dolomitic sand from a quarry near Jefferson City, Missouri, and the five remaining plots were filled with mine tailings from Desloge, Missouri, which contained high concentrations of lead, zinc and other heavy metals (Table 1) Fill materials were added to a depth of 15-20 cm Plots were located on a slight slope with PVC collection pipes across the narrow downhill end Openings in these pipes were covered with fiberglass screening and layers of gravel to exclude fill materials

Leachate formed from rainwater percolating through the plots drained through the collection pipes into rigid vinyl pools (1 5 meter diameter, approximate capacity 450 liters) Pipes feeding into the pools were upturned at a right angle to retain leachate samples and encourage settling of suspended solids Natural rainfall was supplemented during a late summer 1983 drought period with ultrasoft water from the CNFRL reverse osmosis apparatus This water was applied to the plots through cotton "soaker hoses" to assure gradual percolation Plots were watered three times between late July and early September with volumes of 200-400 liters to

The research presented here is part of a project funded by the U S Fish and Wildlife Service, Columbia National Fisheries Research Laboratory, to examine the effects of vegetation and organic cover materials on the mobilization, biological availability and toxicity of metals in leachates from Old Lead Belt mine tailings A previous report described the effects of cover materials on the concentration and chemical forms of metals leached from tailings test plots (Harwood 1984) This thesis presents data on metal bioaccumulation, survival, growth and development in aquatic invertebrates exposed to leachates from these test plots Although this project was intended primarily to investigate environmental effects of mine tailings reclamation efforts, the results of the chemical and biological studies also provided an opportunity to examine the dynamics and toxicity of heavy metals in a simplified freshwater system replace evaporative losses from the pools

Efforts were made to assure adequate conditions in the pools for invertebrate bioassays A layer of chert gravel about 2 cm deep was added to the pools A battery-powered compressor provided aeration during the summer months A black vinyl mesh cover erected over the six pools provided shade and helped moderate summer water temperatures A waterproof coating was applied to the shade cover to prevent dilution of leachate water with rainfall

Tailing Cover Treatments

Cover materials were added to the test plots in spring and summer 1982 to simulate a range of conditions for leachate formation The <u>Control</u> plot received no additional cover materials The tailings plots received the following cover treatments

<u>Uncovered</u> Tailings in this plot were left uncovered to approximate the existing condition of the Old Lead Belt tailings piles Virtually no plant growth was established on this plot during the course of the study

<u>Seed</u> This plot was fertilized using a commercial lawn fertilizer (Scott's Starter, 18-24-6% N-P-K) using label directions and seeded with a grass and legume seed mixture This treatment is similar to procedures previously used to revegetate the tailings deposits (Novak and Hasselwander 1980) Vegetation on this plot grew vigorously during moist weather and died back

Table 1 Chemical composition of tailings used in test plots (from Harwood 1984)

	Plot				
	Untreated	Seed	Sod	Sludge	Leaf
Major constituents (%)					
Ca	19	21	20	20	20
Mg	9 4	11	7 1	10	11
Fe	3 7	3 8	3 2	3 5	3 6
K	0 51	0 52	0 48	0 67	0 68
Mn	0 43	0 45	0 34	0 38	0 39
Al	0 36	0 45	0 66	0 59	0 56
Minor constituents (ppm)					
Pb	1400	1500	1300	1400	1600
Zn	1000	1000	1300	1400	1400
Na	310	440	310	410	280
Cu	120	160	98	100	100
Sr	51	53	48	54	52
Ni	49	69	17	33	28
Ba	33	45	33	51	44
Cd	24	22	18	28	31

Chemical characteristics of tailings leachates were strongly affected by cover materials, but differences among treatments were less pronounced after equilibration in collection pools (Table 2) Reductions in hardness and increases in pH occurred in pool waters in all treatments Leachate and pool water chemistry in the uncovered and vegetation (seed and sod) treatments was generally similar to that in the control However, leachate from the seed treatment was less alkaline and had higher concentrations of nutrients and organic carbon Organic cover treatments (sludge and leaf) had greatest effects on tailings leachate composition Leachates in these treatments were high in hardness and organic carbon In pool waters, hardness decreased to low levels and pH and alkalinity remained lower than other treatments Pool water in the sludge treatment was higher in nutrients, softer and less alkaline than other treatments Dissolved organic carbon concentrations decreased in pool waters in the sludge treatment but remained very high in the leaf treatment

Metal concentrations in leachate and pool water (Table 3) generally paralleled differences in water chemistry among treatments In the control, metal levels were low or below detection limits in leachate, but lead and cadmium concentrations in pool waters were higher, suggesting external metal inputs The uncovered treatment had metal levels higher than the control, but low relative to other treatments Leachate and pool concentrations of cadmium, lead, and zinc were elevated in the sod treatment, and the during midsummer

Sod The second vegetation treatment recieved a layer of bluegrass sod with its associated topsoil (depth approx 2 cm), to represent an established vegetative cover Vegetation on this plot was more dense than the seed plot but showed similar seasonal growth patterns

Sludge Dried sewage sludge from the Columbia, Missouri, sewage treatment plant was applied in a layer of approximately 4 cm to this plot Dried sludge has been widely used as a fertilizer and mulch to encourage revegetation of materials low in organic matter such as the mine tailings (Novak and Hasselwander 1980) This plot developed a dense grassy sod which was less affected by drought conditions than the seed or sod plots

Leaf The tailings in this plot were covered with a layer of dried silver maple (*Acer saccharinum*) leaves to examine the effects of naturally-occurring organic cover materials on leachate characteristics A second layer of leaves was added in early 1983

Water Chemistry

Water chemistry analyses were performed at the University of Missouri Environmental Trace Substances Research Center, Columbia, Missouri Data on leachate and pool water chemistry reported here refer to analyses performed by Harwood (1984) on samples filtered through membrane filters with 0 45 micrometer nominal pore diameter

Table 3 Metal concentrations in filtered leachate and pool water Values in first row for each metal are means of pool samples, second row values are leachate means Units are micrograms/liter except Zn and Mn, mg/liter Data from Harwood (1984) Asterisk indicates one or more samples below detection limit, these values set at one-half detection limit for calculations

Metal	Treatment					
	Control	Uncovered	Seed	Sod	Sludge	Leaf
N	4	5	5	5	6	8
	2	5	5	6	5	6
Cd	0 21*	0 32*	0 64	0 71	0 38*	1 06
	<0 2	5 0	14 4	9 7	10 86	7 45
Cu	<10	<10	11 6*	<10	17 2*	11 1*
	<10	<10	45 6	12 0	75 2	24 5
Mn	0 6*	0 7*	1 4*	0 7*	2 6*	49 3
	2 0	1 4	10 2	6 3	8 4	440
Ni	<10	6 8*	18 6	11 8*	10 3*	18 3*
	<10	16 0*	103	29 0	33 2	108
Pb	1 7*	1 9*	1 7*	2 6*	1 7*	13 2
	0 7*	8 2	3 9	17 6	11 2*	48 8
Zn	<0 01	0 03*	0 04*	0 08	0 02*	0 24
	<0 01	1 26	2 06	2 09	1 28	4 03

Table 2 Chemical characteristics of filtered leachate and pool water samples collected May-October 1983 Values listed in first row for each parameter are means of pool samples, second row values are leachate means Units (except pH) are mg/liter Data are from Harwood (1984) Asterisk indicates one or more samples below detection limits, these values were set at one-half detection limit for calculations

Parameter	Treatment					
	Control	Untreated	Seed	Sod	Sludge	Leaf
N	4 2	5 4	5 4	5 5	6 4	8 5
pH	8 01 7 45	8 24 7 18	8 00 6 87	8 14 7 12	7 65 7 05	7 76 7 17
Total Alkalinity (as $CaCO_3$)	95 77	78 106	77 88	87 135	52 42	69 180
Ca + Mg Hardness (as $CaCO_3$)	114 187	109 189	121 180	116 170	97 239	102 253
Nitrate	<5 36 0	2 6* 10 4	14 5* 40 2	2 7* 10 3	27 4* 91 3	10 2* 21 2
Phosphorus	0 073* <0 05	0 128 0 112*	0 158 1 030	0 160 0 046*	0 290 0 540	0 104 0 192
Organic Carbon	3 3 4 5	3 1 1 6	7 4 14 0	4 1 4 3	9 6 20 4	15 1 14 0

METHODS AND MATERIALS

Invertebrate Exposures

Three benthic macroinvertebrates were chosen for leachate exposures based on their similarity to typical Ozark stream fauna, their suitability for use in bioaccumulation and chronic toxicity studies, and their availability from local sources Several species of crayfish have been used in field and laboratory studies of metal bioaccumulation and toxicity (Hubschmann 1966, Vermeer 1972, Thorp et al 1979, Anderson and Brower 1980, Knowlton et al 1983) Orconectes nais, a species occurring in streams of the Ozarks and southern plains (Hobbs 1976), was obtained from a population in an experimental pond on the CNFRL grounds Young-of-the-year O nais were used in leachate exposures because of their relatively small size and their rapid growth through a series of 6-10 molts during their first growing season One hundred crayfish with mean rostral-carapace length of 10 0 mm were stocked in each pool in early July 1983, and allowed to range freely in the gravel substrate of the pools during a 120-day exposure All crayfish were collected and counted after 30 and 60 days exposure and rostral-carapace length was measured on a random subsample of 25 individuals On each sample date, five to ten crayfish from each pool were frozen in polyethylene bags for later metal analysis All surviving crayfish were collected, counted, and measured after 120 days Crayfish from each pool were then randomly sorted seed treatment had relatively high levels of all metals except lead Despite the effects of the sludge treatment on leachate and pool water chemistry, metal concentrations were generally not high in this treatment Leachate from the sludge treatment was high in copper and cadmium, but pool water had low concentrations of most metals The highest overall metal concentrations occurred in the leaf treatment The leachate from this treatment had much higher concentrations of zinc, lead and manganese than other treatments and pool water had highest concentrations of all metals except copper and nickel commercial pet food recommended by Biever (1965) A preliminary 30-day exposure was conducted in the leachate pools in October 1983, with two replicate containers per pool Additional midge exposures were conducted in the laboratory in November-December 1983 due to low water temperatures and slow midge growth rates in leachate pools Laboratory exposures of 10 and 25 days duration were conducted using pool water from four treatments (control, uncovered, sod and leaf), with three replicates per treatment in each exposure Water in the laboratory exposures was replaced daily with fresh (less than 24 hours after collection) pool water and food suspension was added at alternate water changes Growth in total length was measured for surviving larvae after pool and lab exposures Additionally, development stage (larval instar, pupa or adult) was recorded for all midges after laboratory exposures Instar determinations were made based on head-capsule width All midge measurements were made using a binocular dissecting scope equipped with an ocular micrometer

Invertebrate Metal Analyses

Frozen invertebrate samples from leachate exposures were divided into composite samples for lead and cadmium analysis consisting of 1-4 crayfish or 1-7 mayflies, depending on the size of individuals Midge larvae from pool exposures were combined into a single composite sample per treatment, while each replicate from lab exposures was into two groups, one of which was frozen intact for metal analysis The remaining crayfish from each pool were held in pool water for four days at five degrees C to allow clearance of gut contents, then frozen for metal analysis

The burrowing mayfly _Hexagenia bilineata_, of recent interest as a bioassay organism (Fremling and Mauck 1980), was also chosen for use in leachate exposures A similar species, _Ephoron album_, occurs commonly in the Big River (Whelan 1983) In late June 1983, nymphs from a pond culture at CNFRL were stocked in the pools in screened enclosures supplied with plastic tube artificial substrates and fed periodically with a suspension of Tetra-Min tropical fish food The nymphs began to emerge during pool exposures and those that remained were collected after 22 days for metal analysis No additional mayfly exposures were attempted due to a lack of suitable early instar nymphs

The midge _Chironomus riparius_, a member of a widespread and ecologically important group, was chosen to replace _Hexagenia_ for additional leachate exposures Eggs and larvae of chironomid midges have been widely used in invertebrate toxicity testing (Thornton and Wilhm 1975, Wentsel et al 1977, Anderson 1980, Anderson et al 1980) and individuals of this species were available from CNFRL to establish a laboratory culture Midges exposed to leachates were held in 0 5 liter plastic containers with fine mesh windows to allow water circulation and provided with a fine sand substrate Thirty second-instar (4-5 day old) larvae were stocked in each container Larvae were fed a per concentration)

Quality assurance procedures were followed during all metal analyses Digestion and analysis of National Bureau of Standards oyster tissue (Standard Reference Tissue #1566) yielded recoveries of 96 9 ± 3 5 and 101 3 ± 5 8 percent (mean ± SE) for 13 lead and 16 cadmium analyses, respectively Replicate analyses of invertebrate and reference tissue samples showed mean percent reproducibility (difference in concentration between replicates divided by mean concentration, expressed as percent) of 12 3% for cadmium (N=23) and 12 0% for lead (N=19) Some procedural blanks showed detectable levels of lead and cadmium, probably due to cross-contamination among samples during ashing No correction was made for mean blank levels of 9 5 nanograms lead and 1 5 nanograms cadmium

Statistical Methods

All statistical data analysis was performed using the Statistical Analysis System (SAS Institute 1982) on the University of Missouri-Columbia computer system Differences in metal bioaccumulation or leachate toxicity among treatments, study species or sampling dates were tested using rank-based nonparametric tests The Kruskal-Wallis test and multiple comparison procedure (Conover 1980) were used for one-way analyses Two-way analyses (e g , treatment X date) were performed in an analogous fashion using a rank transformation prior to applying two-way analysis of variance and Duncan's new multiple range test analyzed as a composite Mean length was determined for each composite Samples were placed in porcelain crucibles, dried at 70 degrees C for 24 hours, weighed, and ashed at 400-450 degrees C for 24 hours in a muffle furnace Ashed samples were acidified with ultrapure 10% nitric acid, remaining solids were pulverized with a polyethylene pipet tip and the acidified samples were transferred to borosilicate glass vials and heated to sub-boiling on a hot plate for approximately ten minutes to aid dissolution of the ash A small amount of insoluble material, apparently ingested sediment, was observed in some whole-body samples All glassware and instruments were acid-washed in 10% nitric acid and rinsed with ultrapure deionized water before use in sample preparation and analysis

Lead and cadmium analysis was performed using a Perkin-Elmer model 5000 atomic absorption spectrophotometer equipped with a microprocessor-controlled HGA-500 graphite furnace The method of standard additions was used to correct for sample matrix interferences Equal volumes of three standard concentrations, prepared from stock solutions containing 0 0, 1 0, and 2 0 micrograms Pb/ml and 0 0, 0 10, and 0 20 micrograms Cd/ml were added to equal aliquots of each sample The volume of standard added and the sensitivity of the instrument were adjusted according to the absorption of undiluted samples Metal concentrations were determined by linear regression of standard concentration on absorbance Regression was performed on six X Y pairs per sample (3 standard concentrations X 2 absorbance readings

BIOAVAILABILITY OF LEAD AND CADMIUM IN TAILINGS LEACHATE

INTRODUCTION

Heavy metals enter aquatic habitats in a variety of forms from both natural and anthropogenic sources and accumulate in the tissues of aquatic organisms (Forstner and Wittman 1980) Absorption from solution over gills and other exposed body surfaces is generally the most important route of metal uptake, but metals can also be mobilized from ingested solids and absorbed in the digestive tract (Patrick and Loutit 1978, Boothe and Knauer 1972) Dissolved metals tend to adsorb to solids in the aquatic environment, including the surfaces of aquatic organisms Adsorbed metals apparently enter aquatic organisms by passive diffusion mediated by binding to protein molecules (Bryan 1971) Because metals tend to accumulate in aquatic sediments, benthic organisms are especially prone to metal bioaccumulation (Anderson et al 1978, Enk and Mathis 1977)

Characteristics of aquatic organisms exposed to heavy metals affect both rates of metal bioaccumulation and the relative importance of different routes of metal uptake Passive absorption of metals over body surfaces is favored by high surface area/mass ratios due to morphological characteristics such as small body size and large external gills (Smock 1983b) Differences in the permeability and chemical composition of body surfaces may also affect metal uptake from water For example, the heavy calcareous exoskeletons of some crustaceans are less permeable to (rank ANOVA/DNMRT, Conover and Iman 1981) The degree of association between invertebrate metal bioaccumulation and metal toxicity and the association of these variables with water metal concentrations were tested using linear regression and Pearson's product-moment correlation coefficient (Snedecor and Cochran 1980) Statements of statistical significance indicate p-values less than or equal to 0 05 unless stated otherwise

(Gillespie et al 1977, Patrick and Loutit 1978, Stinson and Eaton 1983) Metal bioaccumulation by macroinvertebrates and other aquatic organisms from streams in southeast Missouri has been related to inputs of heavy metals from lead mining activities (Gale et al 1973, Schmitt and Finger 1982, Whelan 1983) High lead concentrations in fish from the Big River, St Francois Co , Missouri, have prompted warnings of possible health hazards to consumers of fish from contaminated reaches of this stream (Czarneski 1980) The Big River and its tributaries receive inputs of metals from erosion of fine-grained lead mine tailings into river channels and solubilization of metals in leachates from large tailings deposits (Kramer 1976, Novak and Hasselwarder 1980)

Measures proposed for stabilization and revegetation of abandoned tailings deposits in the Big River drainage (Novak and Hasselwander 1980) may affect the mobilization of metals in tailings leachates and their availability to aquatic organisms in the Big River system Physical, chemical and biological processes associated with tailings cover materials could affect the predominant forms of leachate metals Reduction in pH of leachate due to decomposition processes in overlying organic materials would increase solubility of inorganic metal compounds such as carbonates and favor release of metals adsorbed to solids (Adams and Sanders 1984) However, high concentrations of calcium and magnesium carbonates would favor formation of complexes and precipitates which could reduce metal availability (O'Shea metals than thinner, less calcified body surfaces such as the cuticle of insects (Bryan 1971, Forstner and Wittman 1980) Metals may be accumulated to high concentrations in the crayfish exoskeleton, but these metals are not readily released to internal tissues and are largely lost during molting (Bryan 1967, Knowlton et al 1983) The importance of metal uptake from ingested sediments may be increased in organisms such as filter-feeders which select fine organic particles high in available metals (Smock 1983a, Whelan 1983)

The chemical speciation of metals affects their bioaccumulation by aquatic organisms Heavy metals are most available as free dissolved ions, but in natural waters a large proportion of total metal concentrations may be present as soluble complexes with organic or inorganic ligands or in precipitated or adsorbed form in suspended solids or bottom sediments (Forstner and Wittman 1980, Moore and Ramamoorthy 1984) Metal complexes are generally less available than free ions, but complexation may enhance metal uptake by maintaining metals in soluble forms under conditions which would otherwise favor precipitation or adsorption (Bryan 1971) Sediment-bound or precipitated metals may be available for uptake by burrowing or detritivorous species (Boothe and Knauer 1978)

Metal concentrations in benthic macroinvertebrates have been studied as indicators of heavy metal pollution (Anderson and Brower 1978, Nehring 1976) and as a pathway of metal accumulation in higher order consumers, including man

Table 4 Mean whole-body lead bioaccumulation (ug/g dry wt ± SD) by three invertebrates during exposures to mine tailings leachates For each sample date, treatments with the same letter are not significantly different (Kruskal-Wallis test with multiple comparisons, $p \leq 0\ 05$)

Crayfish

	Days of Exposure		
	30	60	120
N	4	3	6
Control	1 7 ± 21 a	2 2 ± 34 a	1 1 ± 17 a
Uncovered	1 7 ± 27 a	4 3 ± 52 b	4 5 ± 62 b
Seed	2 4 ± 17 b	4 1 ± 26 b	3 9 ± 31 b
Sod	3 9 ±1 1 b	5 0 ±1 1 b	7 9 ± 86 c
Sludge	12 2 ±5 2 c	19 9 ±4 2 c	14 9 ±2 8 d
Leaf	21 2 ±2 3 c	46 3 ±5 3 c	32 1* ±8 2 e

Mayfly and Midge

	Species (days of exposure)		
	Mayfly (22)	Midge (10)	Midge (25)
N	3	3	3
Control	16 7 ±8 5 a	4 3 ± 12 a	—
Uncovered	24 9 ±7 5 ab	5 5 ± 18 b	5 1 ± 85 a
Seed	24 4 ±6 0 ab	—	—
Sludge	35 0 ±3 8 bc	—	—
Sod	37 0 ±3 3 bc	7 8 ±1 0 c	7 3 ± 40 b
Leaf	193 3 ±30 c	13 5 ±1 4 d	20 4 ± 61 c

* =5

and Nancy 1978) Dissolved organic compounds such as fulvic acids leached from organic detritus or formed by microbial decomposition increase metal solubility due to their ability to bind metals in metal organic complexes (Ramamoorthy and Kushner 1978)

This study examined the biological availability of lead and cadmium in leachates from lead mine tailings under several cover material treatments, including uncovered tailings, vegetation and organic mulches (See Chapter 2) Aquatic invertebrates representative of the Big River fauna were exposed to tailings leachates to address these objectives

(1) determine lead and cadmium bioaccumulation by aquatic invertebrates from mine tailings leachates generated under different cover material treatments, and

(2) evaluate the importance of abiotic and biotic influences on the bioavailability of heavy metals in mine tailings leachates

RESULTS

Cover Treatment Effects

Whole-body lead concentrations in young-of-the-year crayfish were significantly different among treatments after 30, 60 and 120 days of leachate exposure (Kruskal-Wallis test, $p \leq 0.05$, Table 4) Multiple comparisons among

(54 ug/g) confirmed this problem, although the treatment differences observed in mayfly samples are still informative Lead concentrations were not obtained for midges from the seed and sludge treatments during laboratory exposures, but larvae from all other tailings treatments accumulated significantly more lead than controls, with the leaf treatment again showing the highest value

Cadmium was generally accumulated to lower levels than lead by all three invertebrate taxa (Table 5) Differences among treatments were also less marked for cadmium, although significant treatment differences were observed for crayfish and midges Crayfish from the uncovered and vegetation treatments had slightly lower cadmium levels than controls in 30-day samples Cadmium levels in crayfish from the uncovered treatment remained lower than controls throughout the exposure Crayfish cadmium concentrations in both vegetation treatments were significantly higher than in the uncovered treatment after 60 days, and crayfish in the seed treatment reached cadmium levels significantly higher than controls in 120-day samples Crayfish from organic cover treatments showed highest cadmium bioaccumulation, with levels significantly higher than other treatments on all sampling dates (except for the seed treatment on day 120) In laboratory exposures, midge larvae from the uncovered tailings treatment also had cadmium concentrations lower than in controls, but cadmium concentrations were significantly increased in larvae from the sod and leaf treatments Mean cadmium levels in mayfly treatments indicated that crayfish from vegetation and organic cover treatments had significantly higher mean lead levels than controls after 30 days and crayfish from all tailings treatments had significantly higher lead levels than controls in subsequent samples Of the vegetation treatments, the sod treatment showed increased crayfish lead concentrations compared to the uncovered treatment but the seed treatment did not Highest bioaccumulation of lead occurred in the organic cover treatments (sludge and leaf), which showed significantly higher mean crayfish lead concentrations than controls or vegetation treatments on all sampling dates Crayfish from the leaf treatment consistently showed highest mean lead concentrations, which were significantly higher than the sludge treatment by the end of the 120-day exposure

Similar trends in lead bioaccumulation were observed for mayfly and midge exposures (Table 4) and differences among treatments were significant for both species Mayfly nymphs showed slight increases in lead concentration compared to controls in the uncovered tailings, seed and sludge treatments, but only nymphs from the sod and leaf treatments showed lead levels significantly higher than controls The lead concentration of 193 ug/g in mayflies from the leaf treatment was the highest invertebrate metal level observed in this study However, the high lead concentration observed in nymphs from the dolomite control (16 7 ug/g) suggested a problem with lead contamination The high lead concentration in pre-exposure mayfly samples nymphs from vegetation and organic cover treatments were higher than controls and uncovered tailings, with a maximum of 7.3 µg/g in the sludge treatment, but cadmium levels in mayfly samples were variable and treatment differences were not significant.

Temporal Variation

Significant changes in metal concentrations over time occurred in midge and crayfish exposures (Rank ANOVA/DMRT). Lead concentrations in midges did not differ significantly between 10- and 25-day exposures. Midges from the leaf treatment showed increased mean lead concentrations in 25-day samples, while other treatments showed slight decreases. Cadmium concentrations in midge larvae from tailings treatments were significantly reduced between 10- and 25-day exposures. The dolomite control was not included in this analysis because all control larvae had either pupated or emerged after 25 days. Lead and cadmium concentrations in crayfish showed significant overall increases between 30 and 60 day samples, but differences between 60- and 120-day means were not significant. Overall, lead concentrations declined and cadmium concentrations increased between day 60 and day 120, but both metals remained significantly higher than 30-day levels.

Temporal changes in crayfish metal concentrations differed among cover treatments (Table 6). In the control, neither lead nor cadmium concentrations in crayfish changed significantly from 30-day levels (Kruskal-Wallis test).

Table 5 Mean whole-body cadmium bioaccumulation (ug/g dry wt ± SE) by three invertebrates during exposures to mine tailings leachate For each sample date, treatments with the same letter are not significantly different (Kruskal-Wallis test with multiple comparisons, $p \leq 0\ 05$)

Crayfish

	Days of Exposure		
	30	60	120
N	3	3	6
Control	2 7 ± 28 a	2 7 ± 84 ab	2 5 ± 37 ab
Uncovered	1 6 ± 69 a	1 4 ± 33 a	1 7 ± 23 a
Sod	2 3 ± 43 a	3 7 ± 65 b	4 0 ± 58 b
Seed	1 6 ± 21 a	4 8 ± 65 b	8 1 ± 43 c
Leaf	4 4 ± 43 b	13 7 ±1 8 c	11 8* ±4 4 c
Sludge	4 9 ± 61 b	11 0 ± 88 c	14 8 ±3 0 c

Mayfly and midge

	Species (days of exposure)		
	Mayfly (22)	Midge (10)	Midge (25)
N	3	3	3
Control	2 4 ±1 3 a	4 0 ± 14 b	--
Uncovered	2 4 ± 56 a	3 0 ± 26 a	1 2 ± 04 a
Sod	2 8 ± 55 a	4 9 ± 16 c	2 4 ± 22 b
Seed	3 9 ± 39 a	--	--
Leaf	5 3 ±1 4 a	5 9 ± 95 a	2 6 ± 07 b
Sludge	7 3 ±3 0 a	--	--

* N=5

Lead concentrations in crayfish increased significantly from 30-day levels in the uncovered and seed treatments after 60 days and in the sod treatment after 120 days Cadmium concentrations in crayfish increased significantly between 30- and 60-day samples in the seed, sludge and leaf treatments, and a further significant increase occurred in the 120-day sample from the seed treatment

Species Differences

Differences in bioaccumulation among the three species were more marked for lead than cadmium (Table 7) Lead concentrations in mayflies (22-day exposure) were significantly higher than concentrations in either crayfish (30-day) or midges (10- or 25-day, rank ANOVA/DNMRT) Lead concentrations were also significantly higher in midges than in crayfish Cadmium bioaccumulation was more similar among all species Cadmium concentrations in midge larvae after after 10-day exposure were significantly higher than mayflies or crayfish, but cadmium concentrations were lower in 25-day midge samples and were not significantly different than other species Crayfish in most treatments eventually accumulated higher cadmium levels than those measured in samples from the midge and mayfly exposures, which were of shorter duration

Routes of Metal Uptake

Differences in invertebrate lead concentrations among

Table 6 Differences in mean crayfish lead and cadmium concentration (ug/g dry wt) among three sampling dates For each treatment, dates with the same letter are not significantly different (Kruskal-Wallis test with multiple comparisons, $p \leq 0\ 05$)

Day (N)	Treatment					
	Control	Uncovered	Seed	Sod	Sludge	Leaf
Lead						
30 (4)	1 7 ab	1 7 a	2 4 a	3 9 a	12 2 a	21 2 a
60 (3)	2 2 b	4 3 b	4 1 b	5 0 a	19 9 a	46 3 a
120 (6)	1 1 a	4 5 b	3 9 b	7 9 b	14 9 a	32 1* a
Cadmium						
30 (4)	2 7 a	1 6 a	1 6 a	2 3 a	4 9 a	4 4 a
60 (3)	2 7 a	1 4 a	4 8 b	3 7 a	11 0 b	11 8 b
120 (6)	2 5 a	1 7 a	8 1 c	4 0 a	14 8 b	13 7* b

* N=5

the cover treatments were strongly correlated with lead concentrations in leachate water Regression of lead concentrations in mayfly and crayfish versus leachate for the six treatments produced significant positive slopes (Figure 1) Similar comparisons of lead concentrations in invertebrates versus pool water were not as instructive, due to the clumping of lead concentrations near analytical detection limits in most treatments (Table 3) Lead bioconcentration factors (BCF), ratios of lead concentration in invertebrates (in ug/g) to pool water (in ug/L), were used as an alternative to correlation analysis (Table 8) The consistency of lead BCF values within each species is striking in light of the range of lead bioaccumulation among treatments and the analytical problems associated with measuring the low lead concentrations in pool water The sludge treatment is an exception, with mayfly and crayfish lead BCF values 50-300% greater than those from other treatments

Treatment differences in invertebrate cadmium bioaccumulation did not correspond closely to cadmium concentrations in pool or leachate water Invertebrate cadmium concentrations were not significantly correlated with cadmium concentrations in water (Figure 1) Cadmium BCF values (Table 8) also indicate the inconsistent relationship between cadmium concentrations in invertebrates and water Large differences in BCF between the leaf and sludge treatments, both of which had high invertebrate cadmium bioaccumulation, suggest that the importance of

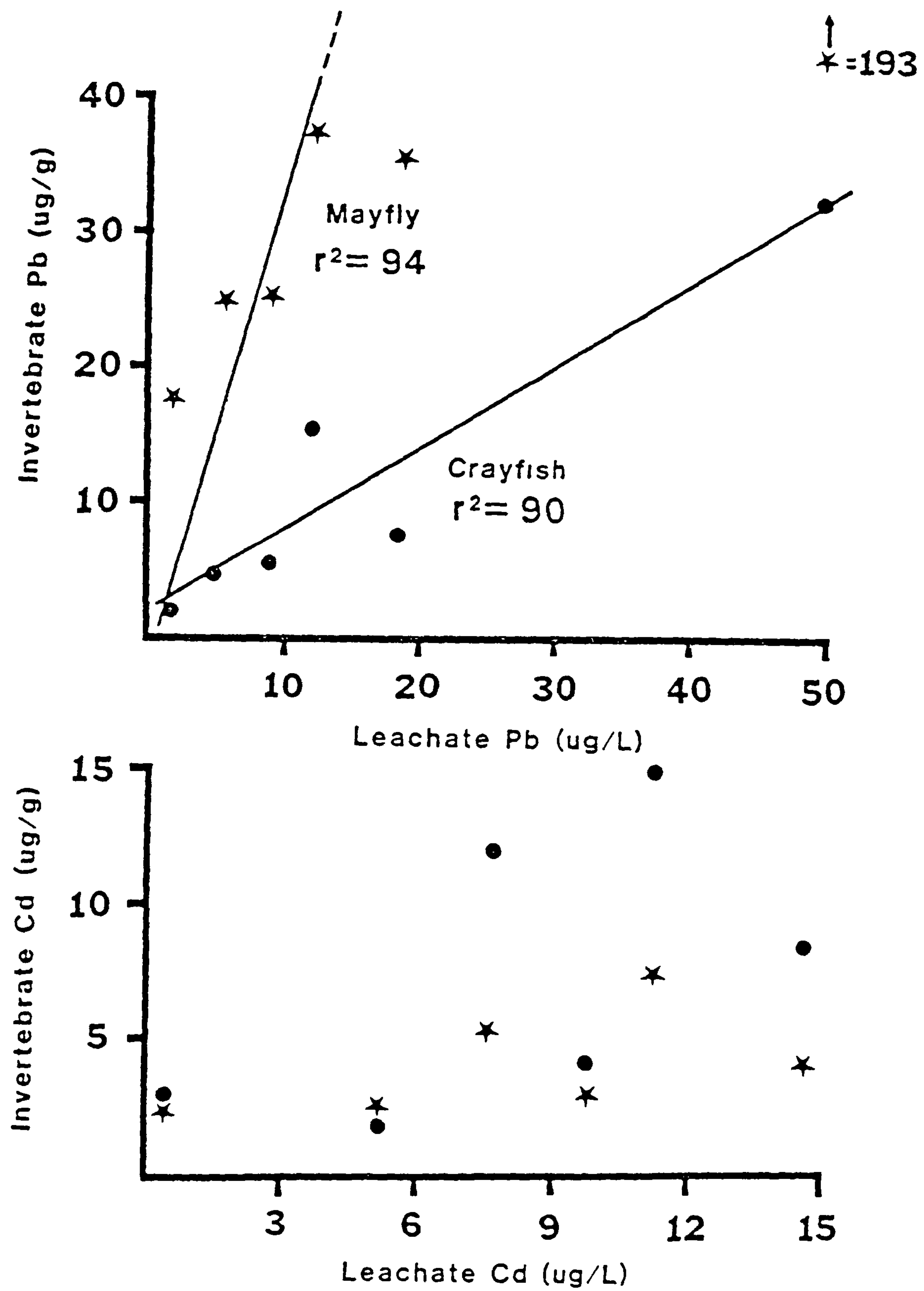
Table 7 Differences in mean lead and cadmium bioaccumulation (N=3) among leachate exposures of three invertebrates For each metal, concentrations in exposures with the same letter are not significantly different (Rank ANOVA with DNMRT, $p \leq 0.05$) Seed and sludge treatments not included in analysis

		Lead		Cadmium
(high)	a	Mayfly (22-day)	a	Midge (10-day)
	b	Midge (25-day)	b	Mayfly (22-day)
	b	Midge (10-day)	b	Crayfish (30-day)
(low)	c	Crayfish (30-day)	b	Midge (25-day)

Figure 1 Relationship between mean invertebrate whole-body lead and cadmium concentrations and mean lead and cadmium concentrations in leachates among cover treatments Stars represent metal levels in mayflies (22-day exposure), circles represent metal levels in mayflies (120-day exposure) Regressions of lead concentrations in invertebrates and leachate are significant at $p \leq 0.01$

Table 8 Bioconcentration Factors (BCF) for lead and cadmium in invertebrates after leachate exposures BCF=mean invertebrate metal concentration (ug/g) divided by mean water metal concentration (ug/L)

	Species (days of exposure)		
	Crayfish (120)	Mayfly (22)	Midge (10)
Lead			
Control	0 6	11 3	2 5
Uncovered	2 3	13 0	2 9
Seed	2 3	14 2	--
Sod	3 0	14 1	3 0
Sludge	8 9	21 2	--
Leaf	2 4	14 6	1 0
Cadmium			
Control	11 8	11 4	19 0
Uncovered	5 3	7 5	9 4
Seed	12 7	6 1	--
Sod	5 7	4 0	7 0
Leaf	3 9	5 0	5 6
Sludge	11 1	19 2	--



★=193
Mayfly
r²= 94
Crayfish
r²= 90
Invertebrate Pb (ug/g)
40
30
20
10
10
20
30
40
50
Leachate Pb (ug/L)
Invertebrate Cd (ug/g)
15
10
5
3
6
9
12
15
Leachate Cd (ug/L)

Table 9 Effects of gut clearance on crayfish lead and cadmium concentration after 120-day leachate exposures Values are mean metal concentrations (ug/g dry wt ±SD) before (=whole body) and after (=gut-cleared) a four day gut clearance period, percent reduction from whole-body levels, and metal concentration (ug/g) in composite fecal sample collected during gut clearance period

	Whole-body conc (N=6)	Gut-cleared conc (N=6)	Percent reduction	Fecal conc
Lead				
Control	1 1 ± 17	0 7 ± 09	36	16
Seed	3 9 ± 31	2 9 ± 45	27	10
Uncovered	4 5 ± 62	3 3 ± 42	27	127
Sod	7 9 ± 86	4 2 ± 44	46	39
Sludge	14 9 ±2 8	5 9 ± 59	61	357
Leaf	32 1 ±8 2*	18 3 ±1 8**	43	20
Cadmium				
Control	2 5 ± 37	2 4 ± 15	4	7
Uncovered	1 7 ± 23	1 6 ± 25	5	36
Sod	4 0 ± 58	3 1 ± 14	23	35
Seed	8 1 ± 43	7 1 ±1 2	13	127
Leaf	11 8 ±4 4*	7 2 ±0 9**	39	32
Sludge	14 8 ±3 0	10 6 ±0 9	28	63

* N=5

** N=3

water cadmium concentrations to invertebrate cadmium uptake between these two treatments

A four-day gut clearance period reduced lead and cadmium concentrations in crayfish after the 120-day leachate exposure (Table 9) Significantly lower lead concentrations were measured in gut-cleared crayfish compared to whole-body samples (rank ANOVA) The proportion of whole-body crayfish lead concentration lost during gut clearance ranged from 27% in the uncovered and seed treatments to 61% in the sludge treatment Lesser reductions in crayfish cadmium concentrations were measured in crayfish after gut clearance, with reductions from whole-body levels ranging from less than 5% in controls to a 39% in the leaf treatment The percentage of whole-body metal concentration lost after gut clearance was higher in crayfish from treatments with high gut-cleared metal concentration, particularly for cadmium

Concentrations of lead and cadmium in crayfish feces collected during the gut clearance period were greater than levels in crayfish tissues (Table 9) Although there was no consistent relationship between fecal and crayfish metal concentrations among the six treatments, feces from control animals contained low concentrations of both metals compared to those from tailings treatments Lead concentrations were very high in fecal samples from the sludge and, to a lesser extent, the uncovered treatment Cadmium concentrations were also high in fecal samples from the sludge treatment, but highest levels occurred in the seed treatment high in leachates in the seed treatment but declined to low levels in the pool High cadmium concentrations in crayfish fecal samples from the seed treatment suggest the importance of cadmium uptake from ingested solids in this treatment Similarly, low cadmium levels in crayfish feces from the sod treatment may explain the lower cadmium bioaccumulation in this treatment despite water cadmium concentrations similar to other treatments

The greatest bioaccumulation of both lead and cadmium occurred in the organic cover treatments (sludge and leaf) Pool water maintained highest concentrations of both lead and cadmium in the leaf treatment Reductions in concentrations of both metals between leachate and pool waters were much less pronounced in this treatment This increased metal solubility in the leaf treatment is probably due to high concentrations of organic ligands Harwood (1984) documented the presence of fulvic acids and high-molecular weight metal complexes in leaf leachate and pool waters Increased metal bioaccumulation in the leaf treatment reflects the high metal concentrations in pool water, although gut metal loadings were also high in crayfish from this treatment In contrast to the leaf treatment, high bioaccumulation in invertebrates from the sludge treatment cannot be attributed to high metal levels in pool water Leachate from the sludge treatment contained elevated lead and cadmium concentrations, but metal concentrations in pool water remained low The less alkaline sludge pool waters may have facilitated uptake of

DISCUSSION

Cover Treatment Effects

Bioaccumulation of metals by invertebrates during leachate exposures indicated significant differences in the biological availability of lead and cadmium from leachates generated by different mine tailings cover treatments Little difference was observed in invertebrate metal concentrations between the control and the uncovered treatment, despite substantially higher metal levels in leachate from the uncovered treatment Background contamination of lead and cadmium apparently occurred in the control pool Concentrations of lead in invertebrates were slightly higher in the uncovered treatment, but cadmium levels were actually higher in the control The relatively high bioavailability of cadmium in the control may represent antagonistic action of other heavy metals on cadmium uptake (Bryan 1971) in the uncovered treatment, where they occurred at higher levels

Significant increases in metal bioaccumulation occurred in the vegetation treatments (seed and sod) High leachate and pool lead concentrations probably accounted for the greater lead bioaccumulation in the sod treatment than in controls, although lead concentrations in gut contents were also high in crayfish from this treatment Contrasting results were observed for cadmium bioaccumulation, which showed significant increases over controls in the seed rather than the sod treatment Cadmium concentrations were apparently affected by differences in chemical speciation of lead and cadmium in pool waters For cadmium, predicted by equilibrium modeling to precipitate under conditions in the pools, the association of high levels in gut-cleared crayfish with high gut loadings suggests cadmium uptake from ingested solids Treatments with greatest reductions in filtrable cadmium between leachate and pool water also exhibited high crayfish fecal cadmium concentrations and high BCF values Apparently, uptake of precipitated or adsorbed cadmium was more important than uptake from water under conditions of very low water cadmium concentrations A similar situation was observed for lead only in the sludge treatment, which showed very low lead concentrations in pool water but high lead bioaccumulation High gut lead loadings and high fecal lead concentrations in crayfish from this treatment support the importance of uptake of lead from ingested solids

Differences in metal bioaccumulation among study species reflect biological influences as well as effects of exposure conditions and metal chemistry Increased lead bioaccumulation in mayflies and, to a lesser extent, midges compared to crayfish may reflect a greater susceptibility for metal uptake from water in these species Whelan (1983) reported similar rankings of lead bioaccumulation for these taxa in samples from the Big River The mechanism for the observed differences may be higher permeability of the insect cuticle compared to the calcareous crayfish exoskeleton, or simply morphological differences such as dissolved metals from these low concentrations However, the importance of metal uptake from ingested materials in this treatment is suggested by results of the gut-clearance experiments High percentages of whole-body lead and cadmium concentrations were lost from crayfish from the sludge treatment during gut clearance, and fecal samples contained elevated concentrations of both metals

Influences on Bioavailability

The results of this study suggest differences in the relative importance of aqueous and solid-phase metal uptake between lead and cadmium Both the significant correlation of invertebrate lead bioaccumulation with water lead concentrations and the consistency of lead BCF ratios indicate the importance of lead uptake from solution No significant correlation was observed between water and invertebrate cadmium concentrations and cadmium BCF values were highly variable among treatments, apparently reflecting variability in the importance of uptake of dissolved cadmium Further support for these differences was provided by computer modeling of metal speciation in pool waters, which predicted lead to be present primarily as dissolved lead carbonate and cadmium as precipitated cadmium carbonate under conditions in the leachate pools (Harwood 1984) Complexation with dissolved organics probably favored uptake of both lead and cadmium from the aqueous phase in the leaf treatment

The importance of metal uptake from ingested solids was small size and/or large external gills which increase surface area and favor surface absorption of metals (Smock 1983b) The differences are consistent with aqueous lead uptake, since elevated levels of lead occurred in mayfly and midge despite the reduced sediment contact of these species due to the use of enclosures Cadmium uptake was apparently less affected by differences in characteristics of the study species Cadmium bioaccumulation showed only minor differences between mayflies and crayfish after comparable exposure periods Cadmium levels were higher in midges from the 10-day exposure than in other species, but declined to lower levels in the larger larvae from the 25-day exposure These differences suggest a surface area effect consistent with uptake of dissolved cadmium, despite low water concentrations In crayfish, which were free-ranging in the leachate pools, cadmium uptake from ingestion of contaminated solids may have been a major contribution to cadmium bioaccumulation

The results of this study indicate that vegetation and organic cover materials can significantly increase the generation of biologically available heavy metals in tailings leachates The processes of solubilization, complexation with dissolved organics, precipitation and adsorption on organic and inorganic solids resulted in high metal bioavailability Dissolved organic compounds such as those present in the leaf leachate increased the solubilization of lead and cadmium from tailings and maintained these metals in solution as metal organic complexes The conditions of leachate formation in the leaching plots used in this study may not be a good approximation of existing conditions in large tailings piles The shallow tailings depth and short contact time of leachates in the tailings plots apparently did not allow readsorption and precipitation of metals, which occurred in the pools Increased tailings depth negated the effects of cover materials on leachates generated in laboratory studies (Harwood 1984) However, formation of metal-enriched leachates does occur under present conditions in the Old Lead Belt tailings piles (Kramer 1976, Harwood 1984) The benefits of revegetation of the large tailings piles, such as reductions in erosion and rainfall infiltration, probably outweigh the effects of cover materials on leaching of metals from the tailings

The increases in metal mobilization observed in this study may be relevant to the status of tailings eroded into stream and riparian habitats of the Big River drainage The massive erosion of tailings from the Desloge tailings pile and the continuing erosion problems at many of the tailings piles assure the presence of a large reservoir of heavy metals which are potentially available to aquatic biota (Schmitt and Finger 1982) These tailings are distributed in relatively thin deposits in direct contact with vegetation, organic detritus and water, conditions similar to those in the leaching plots The mobility and biological availability of metals from eroded mine tailings is indicated by high metal concentrations in invertebrates and fish from the Big River as far as 90 kilometers downstream from tailings inputs (Schmitt and Finger 1982, Whelan 1983, Czarneski 1984) Heavy metal contamination of the Big River system from both erosion and leaching of tailings deposits may pose a threat of metal toxicity to aquatic organisms as well as a health concern to human consumers of metal-contaminated fish

CHRONIC TOXICITY OF TAILINGS LEACHATE

INTRODUCTION

The toxicity of heavy metals to fish and aquatic invertebrates has been widely documented in laboratory studies Standardized toxicity tests have been used to determine metal concentrations causing 50% mortality or immobilization (the median lethal or "effective" concentration, LC_{50} or EC_{50}) in aquatic organisms in acute (less than 100 hour) exposures to heavy metals (Table 10) Acute metal toxicity to aquatic organisms results from the destructive action of metals on exposed body surfaces Precipitation of membrane proteins on gills and other respiratory surfaces can prevent oxygen uptake, causing death by hypoxia (Bryan 1971) Metals also have significant adverse effects on aquatic organisms during chronic exposures to metal levels much lower than those causing acute lethality (Table 11) At these low concentrations, metals are known to inhibit or block enzyme systems, resulting in increased long-term energy demands and a general decline in fitness (Bryan 1971, Thorp et al 1979) Reduced survivorship and growth, delayed development and impairment of reproduction have been observed in organisms under chronic metal stress (United States Environmental Protection Agency (USEPA) 1980a-e) Chronic metal toxicity may significantly affect population parameters such as rates of population increase (Marshall 1980) and biomass production (Borgmann et al 1978)

Table 10 Selected LC_{50} or LC_{50} values for toxicity of metals to aquatic invertebrates and fish in acute (<100 hour) exposures Ranges of values indicate influence of hardness (low hardness-high hardness) Units are mg/liter

Test Organism	Metal				
	Lead	Cadmium	Zinc	Nickel	Copper
Cladoceran, _Daphnia magna_	0 45-1 91*	0 01-0 07	0 10-0 66	0 51-2 34	0 01-0 20
Amphipods, _Gammarus_ spp	0 12	0 70	8 1	4 0	0 20-0 41
Crayfish, _Orconectes rusticus_					3 0
Snails, _Physa gyrina_		1 4		14 3	
other			0 60-4 4		0 39-1 7
Midge, _Chironomus_ sp		1 2	18 2	8 6	0 30
Mayfly, _Ephemerella_ sp		2 0-28		4 0	
Trout, _Salmo gairdneri_	1 0-542	1 0-6 0	0 90-7 2	35 5	0 20-0 89
Minnow, _Pimephales promelas_	2 0-482	0 63-74	0 60-36	5 0-32	0 23-1 5
Sunfish, _Lepomis macrochirus_		2 0-21	3 0-42	5 0-40	0 66-10

* Values cited from USEPA (1980a-e) water quality criteria documents

Table 11 Metal concentrations causing chronic toxicity in aquatic invertebrates and fish Range of values indicates influence of hardness (low hardness-high hardness) All concentrations in ug/liter

Organism	Metal				
	Lead	Cadmium	Zinc	Nickel	Copper
Cladoceran, *Daphnia magna*	12-128*	0 15-0 44	47-136	14 8-354	9 5-29
Amphipod, *Gammarus pseudolimnaeus*					6 1
Crayfish, *Cambarus latimanus*	10[1]				
Orconectes rusticus					30[2]
Snails, *Lymnaea palustris*	25[3]				
other					10 9
Midge, *Tanytarsus* sp [4]	258	3 8	36 8		16
Trout, *Salmo gairdnerii*	19-102		207	350	19
Salvelinus fontinalis	1 7-9 2				
Minnow, *Pimephales promelas*		46	106	109-527	14-28
Sunfish, *Lepomis macrochirus*	92	50			29

* Values cited from USEPA (1980a-e) unless otherwise indicated 1=Thorp et al 1979, 2=Hubschmann 1966, 3=Borgmann et al 1978, 4=Anderson et al 1980

Sensitivity to chronic metal exposure is affected by physiological or life history characteristics which may enhance or inhibit the toxic action of metals Resistance to metal toxicity can vary considerably with differences in age or life stage, or effects may become apparent during periods of physiological stress such as molting, metamorphosis or reproduction (Bryan 1971, Forstner and Wittman 1980) The toxicity of absorbed metals may be reduced by a variety of processes In freshwater crustaceans, metal toxicity is reduced by complexation with blood proteins and storage and excretion by organs such as the hepatopancreas (Bryan 1971, Wright 1980) or through absorption to the exoskeleton and loss during molting (Wright 1980, Knowlton et al 1983)

The toxicity of metals is strongly influenced by physicochemical conditions in natural waters which affect the proportion of total metals present as free metal ions, the form largely responsible for metal toxicity Reduction in metal ion concentration and metal toxicity can result from complexation with inorganic or organic ligands (Freedman et al 1980, Sunda et al 1978) Thus metal toxicity can be reduced in hard water (Davies et al 1976) or water with high concentrations of dissolved organic compounds (Brown et al 1974, Giesy et al 1979) Metal toxicity can also be reduced by adsorption of metal ions on suspended sediments (Hongve et al 1980, Schuytema et al 1984) Reduced pH increases metal toxicity by favoring release of free metal ions from complexed or adsorbed forms

(O'Shea and Mancy 1978, Adams and Sanders 1984) In contrast to the generally reduced toxicity of bound metals, increases in cadmium toxicity have been reported in the presence of humic compounds (Giesy et al 1979, Gjessing 1981, Winner 1984) Interactions among metals can cause the toxicity of metal mixtures to differ from the simple additive toxicity of individual metals Both antagonism (reduction in toxicity, Eaton 1973, Spehar et al 1979) and synergism (greater than additive toxicity, Brown and Dalton 1970, Hale 1977, Borgmann 1980) have been reported in toxicity studies of metal mixtures

Despite many reports of metal contamination, evidence for toxicity of metals to aquatic organisms in natural systems is limited Studies of streams in Wales attributed impoverished fish, invertebrate and plant communities to toxic metals from lead and zinc mine waste deposits (Carpenter 1924, Johnson and Eaton 1980) The distribution of benthic invertebrates in Palestine Lake, Indiana, was related to differences in sediment metal contamination (Wentsel et al 1977a) Shifts in insect community structure were reported in metal-contaminated stream reaches in Ohio (Winner et al 1980) In the Old Lead Belt of southeast Missouri, low taxonomic diversity of benthic invertebrates and absence of intolerant taxa have been reported in the Big River and Flat River Creek (Ryck 1974, Duchrow 1983), but potential effects of metal toxicity in these streams may be masked by physical impacts of sedimentation of eroded tailings

The toxicity of leachates from mine tailings deposits in the Old Lead Belt has not been evaluated Processes of weathering, oxidation and leaching within the Elvins, Missouri, tailings pile have resulted in high concentrations of lead, zinc and other heavy metals in seepage water entering Flat River Creek (Kramer 1976) The mobilization of metals in tailings leachates may be affected by present land-use practices or by proposed measures for the stabilization and revegetation of the tailings piles (Novak and Hasselwander 1980) Changes in leachate chemistry associated with plant growth, microbial decomposition and increased concentrations of dissolved organic compounds could favor the solubilization of metals from tailing deposits and increase the toxicity of tailings leachates

This study evaluated the potential toxicity of leachates from tailings deposits to aquatic biota in the Big River drainage The design of the leachate generation plots minimized the influence of solid-phase tailings on invertebrate responses, and cover material treatments were chosen to simulate a range of conditions for leachate generation under both existing conditions and proposed reclamation schemes for the tailings piles Chronic leachate exposures using invertebrates representative of the Big River fauna were conducted to address the following objectives

(1) determine the chronic toxicity of mine tailings leachates to benthic invertebrates,

(2) relate effects on invertebrates to differences in the concentration and bioavailability of leachate metals among tailings cover material treatments, and

(3) evaluate potential leachate effects on invertebrate populations in contaminated habitats

RESULTS

Crayfish Survival and Growth

Survivorship of young-of-the-year <u>Orconectes nais</u> in the leachate pools differed among the treatments during the 120-day exposure (Fig 2) Survivorship in the dolomite control was higher than in four of the five tailings treatments on day 30 and higher than all tailings treatments on succeeding sample dates After 120 days, survivorship in the control (57%) was twice as great as the average of the five tailings treatments (29%) Survivorship was lowest in the leaf treatment on all three dates, reaching 13% after 120 days Although mortality patterns differed temporally in the uncovered, seed, and sod treatments, survivorship in these treatments converged at 28-33% after 120 days Survivorship in the sludge treatments did not differ substantially from the control until the 120-day sample, when it reached 41%

Crayfish survivorship showed significant negative linear correlations with mean filtrable heavy metal concentrations in leachate and pool water samples (Table

Figure 2 Crayfish survivorship during 120-day exposure in tailings leachate pools Percent survival was adjusted to account for sample removal

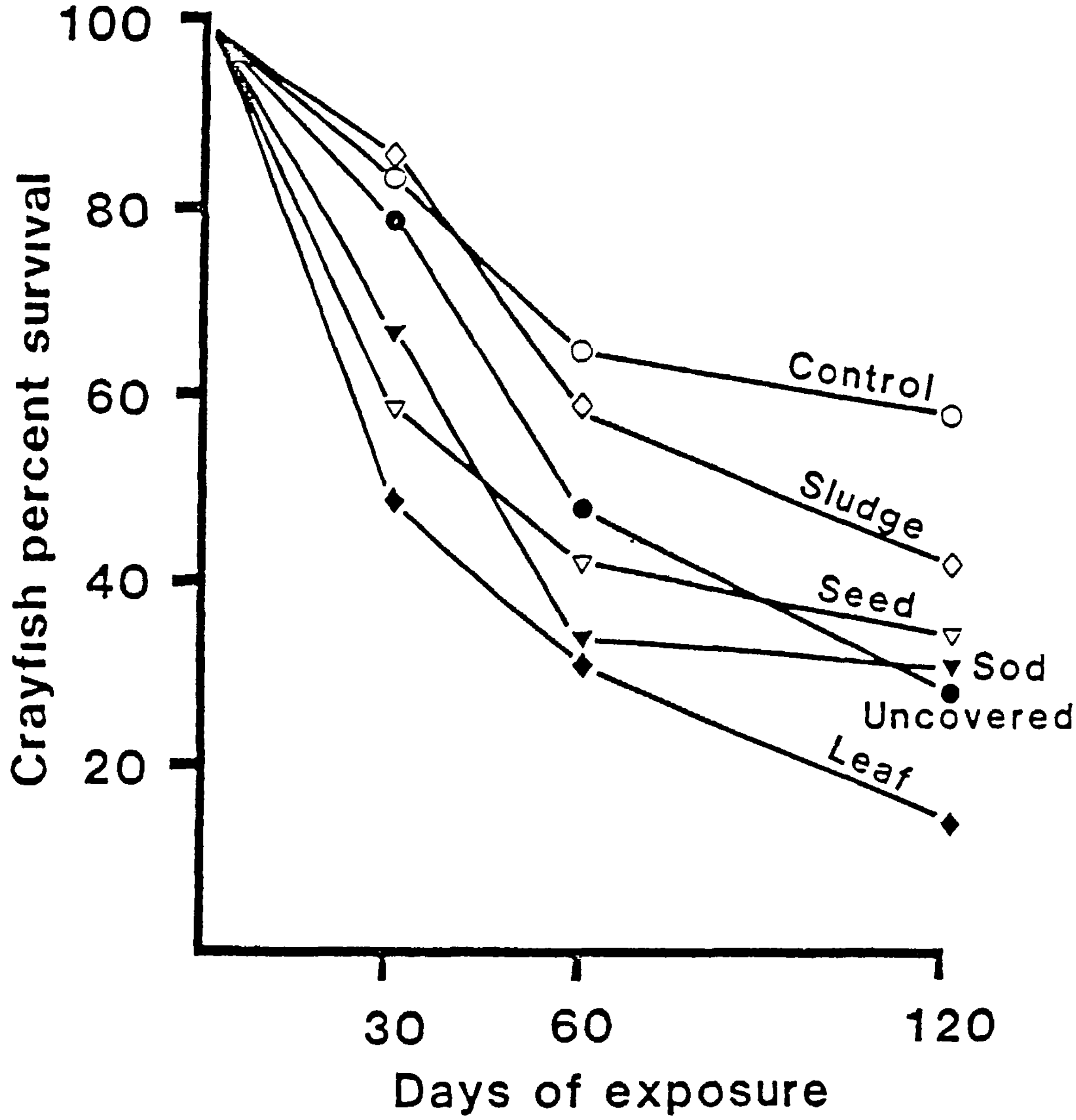

100
80
60
40
20
Crayfish percent survival
30
60
120
Days of exposure
Control
Sludge
Seed
Sod
Uncovered
Leaf

Table 12 Pearson product-moment correlations (N=6) of crayfish survivorship with mean filterable metal concentrations in pool and leachate water and crayfish samples One asterisk indicates $p \leq 0$ 0, two asterisks indicate $p \leq 0$ 05

Day	Metal					
	Pb	Cd	Zn	Mn	Ni	Cu
Survivorship vs pool water metal concentrations						
30	-0 72	-0 92**	-0 82**	-0 70	-0 90**	-0 09
60	-0 59	-0 89**	-0 74*	-0 53	-0 73*	0 09
120	-0 72	-0 84**	-0 80*	-0 69	-0 67	-0 11
Survivorship vs leachate metal concentrations						
30	-0 69	-0 45	-0 90**	-0 71	-0 90**	0 04
60	-0 68	-0 47	-0 87**	-0 55	-0 63	0 17
120	-0 80*	-0 47	-0 92**	-0 70	-0 66	0 03
Survivorship vs crayfish lead and cadmium concentrations						
30	-0 48	0 03				
60	-0 41	-0 25				
120	-0 70	-0 28				

(2) Of the heavy metals measured, only copper failed to show consistently negative correlations with survivorship Zinc, cadmium and nickel concentrations in pool and/or leachate samples were significantly correlated with crayfish percent survival on one or more sampling dates Correlations of lead and manganese with crayfish survival were also strong (p=0 06 for lead, p=0 11 for manganese, Correlations of most metals with crayfish survival were similar for pool and leachate samples, but cadmium correlations were significant only for pool samples Crayfish survivorship was more strongly correlated with body burdens of lead than cadmium

Growth of crayfish also varied greatly among treatments (Figure 3) After 120 days, crayfish from the sod and leaf treatment showed the greatest mean dry weight (>260 mg) Growth was reduced in the tailings and seed treatments (approx 200 mg) and the least growth occurred in the sludge treatment and control (approx 100 mg) Growth did not show negative correlation with metal concentrations in water or invertebrate tissue Growth rates were generally highest during the second sampling interval (30-60 days) and all treatments showed reduced growth during the last interval (60-120 days)

Crayfish growth showed a consistent inverse relationship with survivorship Differences in cumulative growth among treatments showed significant negative correlations with survivorship after 60 and 120 days (Table 13) Interval growth and survivorship were strongl

Figure 3 Growth of crayfish during 120-day exposure in tailings leachate pools Mean dry weight calculated from carapace length dry weight regression, N=25 per treatment

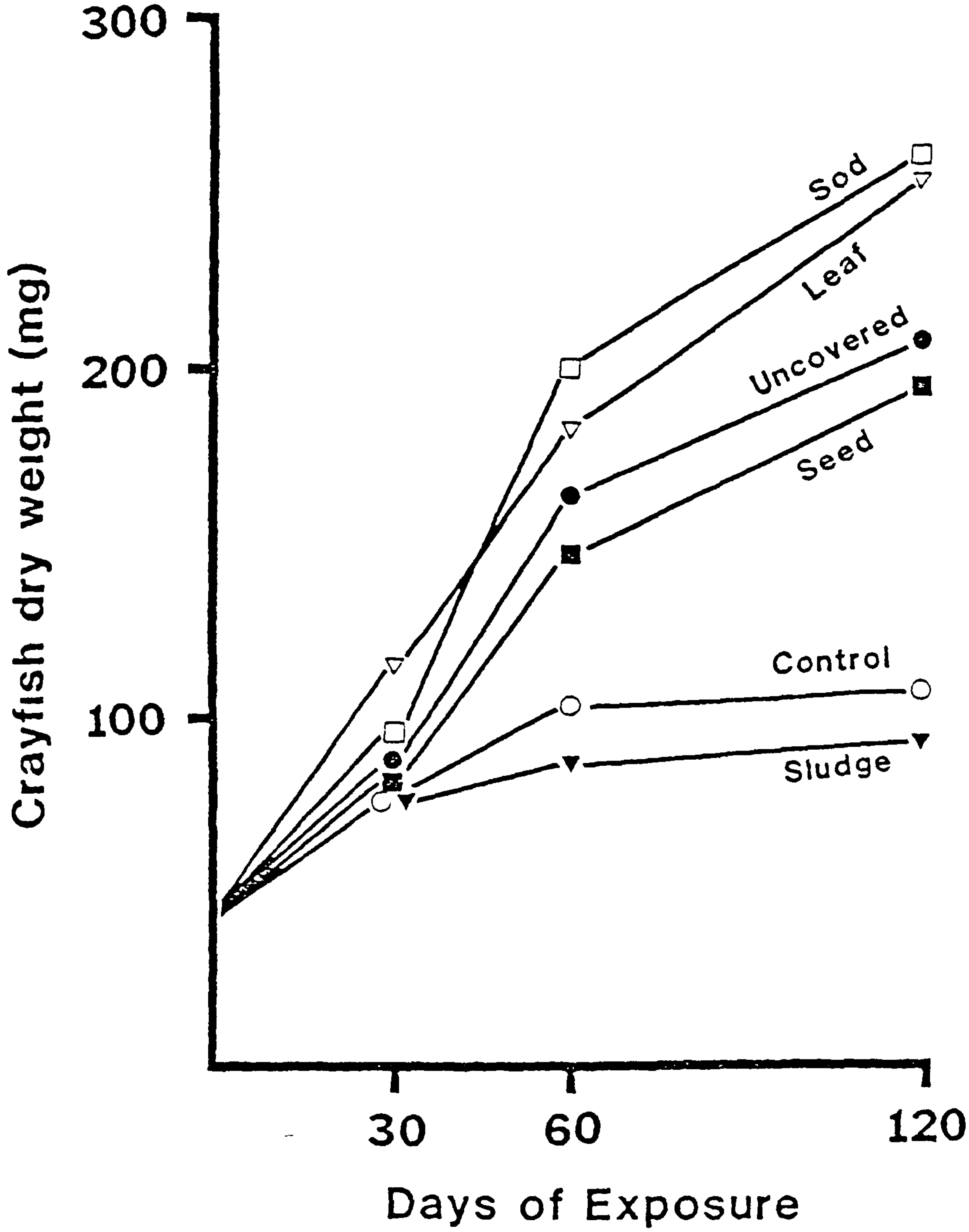

Crayfish dry weight (mg)
300
200
100
Sod
Leaf
Uncovered
Seed
Control
Sludge
30
60
120
Days of Exposure

Table 13 Pearson product-moment correlation (N=6) of crayfish interval and cumulative growth with interval and cumulative survivorship during three sampling intervals Growth in mean dry weight calculated from length weight regression One asterisk indicates $p \leq 0$ 10, two asterisks indicates $p \leq 0$ 05

Interval (days)	Survival	Growth	
		Interval	Cumulative
I (0-30)	Interval	-0 75*	--
II (30-60)	Interval	-0 75*	--
	Cumulative	--	-0 92**
III (60-120)	Interval	-0 39	--
	Cumulative	--	-0 83**

correlated (p<0 10) during the first two sampling intervals The inter-relationship of survivorship and growth is further supported by differences among sampling intervals During the second interval, reduced overall survivorship was associated with highest growth rates The survivorship growth correlation was strongest after the second interval and survivorship during this interval was poorly correlated with water metal concentrations (Table 14)

Midge Survival, Growth and Development

Midge (Chironomus riparius) larval survivorship was differentially affected among cover treatments during the 30-day exposure in the leachate pools (Table 15) Survivorship was over 90% in the control and the uncovered treatment and was only slightly reduced in the seed and sludge treatments Lowest survivorship was observed in larvae in the sod (75%) and leaf (58%) treatments Midge survival showed significant negative correlations with leachate and/or pool concentrations of all metals except nickel and copper (Table 16) Correlation of survivorship with midge lead and cadmium bioaccumulation was also strong (p<0 10)

Larval growth during pool exposures was strongly affected by tailings leachates (Table 15) Mean increase in larval total length was reduced in all tailings treatments compared to the dolomite control Greatest reductions in midge growth occurred in the leaf and sod treatments, to

Table 14 Pearson product-moment correlation (n=6) of crayfish interval survivorship (percent survival/sampling interval) with mean metal concentrations in filtered pool and leachate water samples One asterisk indicates p≤0 10, two asterisks indicate p≤0 05

Interval (days)	Metal Pb	Cd	Zn	Mn	Ni	Cu
Crayfish interval survivorship vs pool water metals						
I (0-30)	-0 72	-0 93**	-0 82**	-0 70	-0 90**	-0 09
II (30-60)	-0 10	-0 36	-0 25	-0 02	-0 05	0 35
III (60-120)	-0 76*	-0 49	-0 70	-0 79*	-0 37	-0 32
Crayfish interval survivorship vs leachate water metals						
I (0-30)	-0 69	-0 45	-0 90**	-0 71	-0 90**	0 04
II (30-60)	-0 33	-0 19	-0 36	-0 03	0 13	0 30
III (60-120)	-0 74*	-0 02	-0 67	-0 78*	-0 50	-0 09

Table 15 Survival, growth and metal bioaccumulation of midge larvae following 30-day exposure in leachate pools Growth and survival are means of two replicates, metal concentrations determined in single composite sample per treatment

	Treatment					
	Control	Uncovered	Seed	Sludge	Sod	Leaf
Survival (%)	90	93	85	83	75	58
Growth (mm)	4 8	2 3	1 7	2 8	1 0	1 3
Pb conc (ug/g)	0 7	3 3	2 9	5 9	4 2	8 3
Cd conc (ug/g)	2 4	1 6	7 1	0 6	3 1	7 1

Table 16 Pearson product-moment correlation (N=6) of midge survivorship and growth during 30-day pool exposure with metal concentrations in pool and leachate water and midge samples One asterisk indicates $p \leq 0$ 10, two asterisks indicates $p \leq 0$ 10

	Metal					
	Pb	Cd	Zn	Mn	Ni	Cu
Midge Survivorship						
Pool	-0 90**	-0 93**	-0 95**	-0 88**	-0 67	-0 26
Leachate	-0 95**	-0 23	-0 89**	-0 88**	-0 64	-0 08
Midge	-0 79*	-0 73*	---	---	---	---
Midge Growth						
Pool	-0 41	-0 79*	-0 57	-0 37	-0 72*	-0 14
Leachate	-0 57	-0 70	-0 82**	-0 37	-0 60	-0 12
Midge	-0 59	-0 52	---	---	---	---

less than 26% of growth in the control. Larval growth was less strongly correlated than was survival with concentrations of most metals in pool or leachate water, and was not significantly correlated with lead and cadmium concentrations in midge larvae (Table 16). Survival and growth showed a nonsignificant positive correlation (r=0.59, p=0.22).

Larval survival was not affected during the 10-day laboratory exposure (Table 17). Survivorship was 98% or greater in all treatments included in the lab study (control, uncovered, sod, and leaf). Larval survivorship remained high in the dolomite control through the 25-day exposure, with 94% of the control larvae reaching the pupal stage. However, reductions in survivorship occurred in the tailings treatments during the longer exposure. Differences in survivorship among treatments were significant after 25 days, and survivorship was significantly lower in all tailings treatments than in controls (Kruskal-Wallis test with multiple comparisons). Mortality during the pupal stage and during emergence was high for both tailings treatments and controls. This mortality was apparently related to development of a surface film caused by the addition of food to the exposure containers rather than leachate toxicity.

Larval growth showed significant differences among treatments in both 10- and 25-day laboratory exposures (Kruskal-Wallis test with multiple comparisons, Table 17). After the 10-day exposure, larvae from all tailings

Table 17 Mean larval survival, growth and metal bioaccumulation (N=3) during ten- and 25-day laboratory leachate exposures) Survival and length means in each row with the same letter are not significantly different (Kruskal-Wallis test with multiple comparisons procedure, $p \leq 0\ 05$)

	Days	Treatment			
		Control	Uncovered	Sod	Leaf
Survival (%)	10	100 a	100 a	98 a	100 a
	25	94 a	48 c	73 b	49 c
Length (mm)	10	11 5 a	8 9 b	9 0 b	7 6 c
	25	-- *	9 4 a	9 1 a	6 6 b
Pb conc (ug/g)	10	4 3	5 5	7 8	13 5
	25	--	5 1	7 8	20 4
Cd conc (ug/g)	10	4 0	3 0	4 9	5 9
	25	--	1 2	2 4	2 6

* No midges remained in larval stage after 25-day exposure

treatments showed significant reductions in total length relative to controls Larval growth was most strongly inhibited in the leaf treatment, which had a 33% reduction in total length relative to controls All surviving midges in the control had reached the pupal stage after the 25-day exposure, but differences in larval length among the tailings treatments were more pronounced than those observed after 10 days Larval total length did not differ significantly between the 10- and 25-day exposures for any treatment (Mann-Whitney tests), but mean length showed slight increases in the uncovered and sod treatments and a reduction in the leaf treatment Larval length in the tailings treatments did not approach that observed in the control after ten days

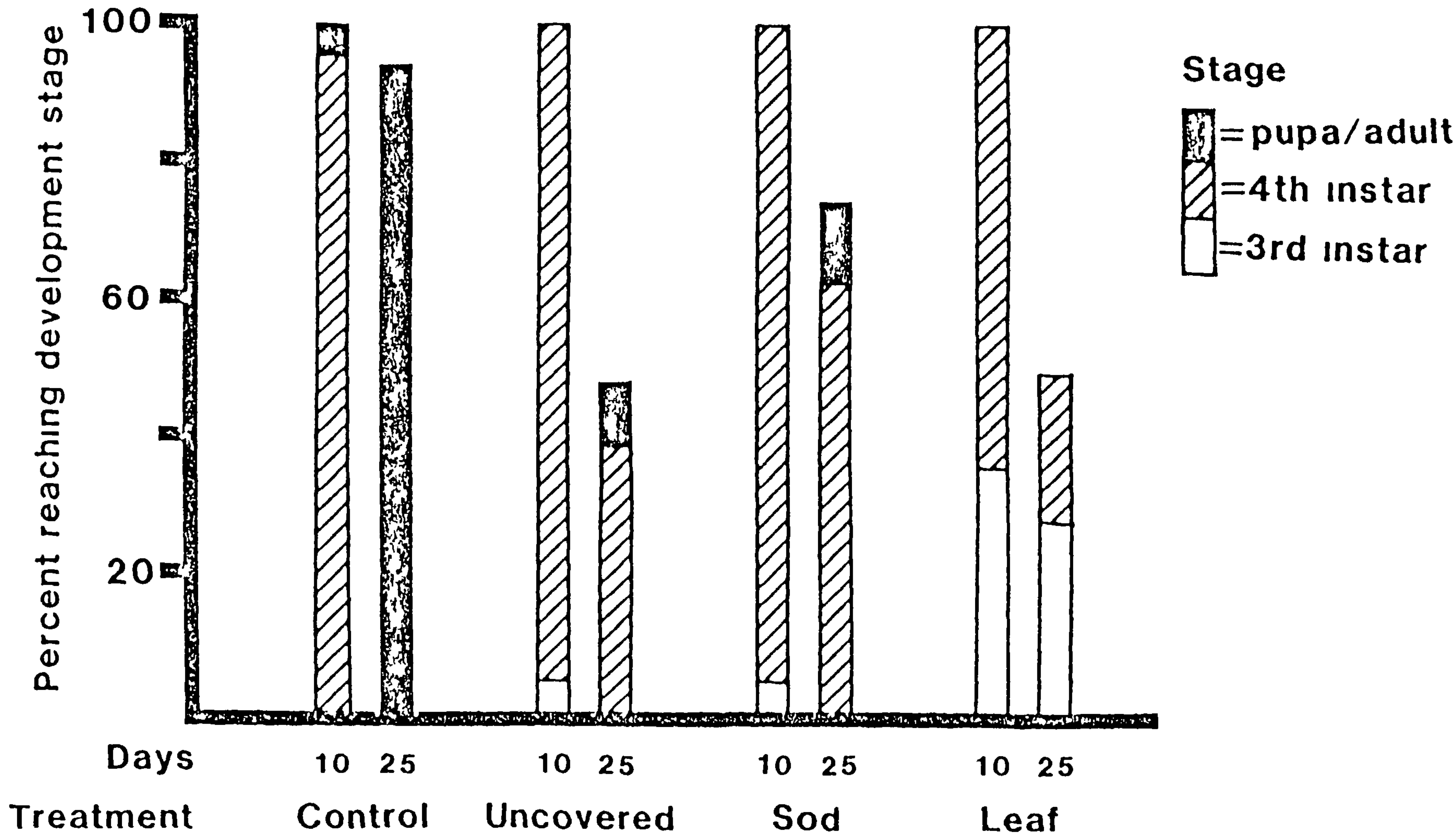
Growth of midge larvae in lab exposures was more strongly correlated with metal bioaccumulation than was survival (Table 18) Total length was significantly negatively correlated with lead concentrations in midge samples from both 10- and 25-day exposures Correlation of total length with cadmium bioaccumulation was strong only in 25-day samples ($p \leq 0$ 10), when no control samples were available

Midge development was delayed or blocked in the tailings treatments (Figure 4) Moderate differences were observed in the 10-day exposure, when most individuals from all treatments were in the fourth (final) larval instar Third instar larvae were still present only in the tailings treatments (35% in the leaf treatment), while midges reached

Table 18 Pearson product-moment correlation of midge survivorship and growth with lead and cadmium bioaccumulation during 10- and 25-day laboratory leachate exposures (N=12 except 25-day growth, N=9) One asterisk indicates $p \leq 0.10$, two asterisks indicates $p \leq 0.05$

	Survival		Growth	
	10-day	25-day	10-day	25-day
Lead	0.10	-0.58**	-0.72**	-0.93**
Cadmium	-0.07	-0.78**	-0.48	-0.62*

Figure 4 Midge development during laboratory leachate exposures Height of bar indicates percent of initial stock reaching each development stage after 10- and 25-day exposures Means of three replicates per treatment on each date


Percent reaching development stage
100
60
20
Days
10 25
10 25
10 25
10 25
Treatment
Control
Uncovered
Sod
Leaf
Stage
=pupa/adult
=4th instar
=3rd instar

the pupal stage only in the control Developmental differences were more pronounced in the 25-day exposure All surviving larvae (94%) in the control had reached the pupal stage, compared 10% and 12% in the uncovered and sod treatments, respectively No pupae or adults were observed in the leaf treatment, in which 30% of the midges remained in the third larval instar after 25 days Emergence was also delayed in the tailings treatments Adults or unsuccessful emergents were observed in the control on days 12-18, while the first emergence occurred on day 15 in the sod treatment and on day 19 in the uncovered treatment

DISCUSSION

Toxic Effects of Leachate

Previous studies with crayfish and midges indicated their sensitivity to metals under conditions similar to the leachate exposures The crayfish Orconectes propinquus was highly resistant to acute cadmium toxicity (Gillespie et al 1977), but exposure to 5-10 ug/L cadmium increased mortality in Cambarus latimanus (Thorp et al 1979) and eliminated populations of Procambarus acutus (Giesy et al 1979) after five month and one year exposures, respectively Adult O virilis were resistant to copper exposure, but newly-hatched young were much more sensitive to copper toxicity (Hubschmann 1966)

Midge larvae may also be resistant to acute but not chronic metal toxicity Acute LC_{50} values for Chironomus sp larvae were over 1 mg/L for cadmium, zinc and nickel, and 30 ug/L for copper (Rehwoldt et al 1973), but chronic (10-day) LC_{50} values for cadmium, zinc and copper for the midge Tanytarsus dissimilis (Anderson et al 1980) were comparable to leachate and pool concentrations of these metals However, these differences may reflect differences in metal tolerance among chironomid taxa Studies of the chironomid community of metal-contaminated streams (contaminated primarily with copper) found Chironomus spp at highly contaminated sites, while Tanytarsus spp occurred only at sites with lower metal levels (Winner et al 1980)

Morphological and life history differences between crayfish and midges may account for the different responses of the two species to leachate metals Reduction in crayfish survivorship without sublethal effects on growth, as seen in leachate exposures, was previously reported in long-term cadmium exposures (Thorp et al 1979) The ability of crayfish to increase growth despite reduced survivorship suggests that crayfish may be only intermittently vulnerable to metal toxicity Although this could result from temporal variation in leachate metal concentrations, crayfish may have become sensitive to metal toxicity only during molts Increased uptake of cadmium by postmolt amphipods, Gammarus pulex (Wright 1980), and shore crab, Carcinus maenas (Wright 1977a), was attributed to changes in calcium regulation occurring during the crustacean molt cycle High cadmium toxicity was associated with increased cadmium uptake in postmolt individuals of both species (Wright 1977b, Wright and Frain 1981) Metals accumulated in the crayfish exoskeleton (Anderson 1978, Knowlton 1983) may be released along with calcium during the premolt phase, further increasing metal exposure of molting individuals These mechanisms would result in pulses of metal toxicity during molts Loss of accumulated metals with the cast exoskeleton and the low metal permeability of the crayfish exoskeleton during intermolt periods would reduce metal exposure, allowing survivors to capitalize on increased food availability

The response of midge larvae to leachate exposure is more consistent with chronic metal toxicity Wentsel et al (1977b) observed sublethal reduction of larval growth in Chironomus tentans exposed to metal-contaminated sediments and speculated that growth reductions indicated impairment of normal development, as was observed in this study in laboratory leachate exposures A noticeable reduction in red pigment in larvae from tailings treatments relative to controls suggested that heme synthesis, known to be affected by lead in vertebrates (Hodson et al 1978), may have been inhibited in midges during leachate exposure Chronic effects were observed in all midge aquatic life stages, suggesting that the relatively permeable midge exoskeleton allowed continuous metal exposure throughout leachate exposures

Influences on Leachate Toxicity

Consistent patterns among tailings cover treatments were evident in leachate and pool water metal concentrations and in the effects of leachate exposure on aquatic invertebrates. Leachate toxicity ranged from the untreated and sludge treatments, which had lowest water metal concentrations in water and reduced effects on invertebrates, through the intermediate seed and sod treatments to the leaf treatment, which had highest metal concentrations and most severe toxic effects.

Several metals were present in leachate and pool water at concentrations exceeding water quality criteria established by regulatory agencies of the United States and Canada (Table 19). These criteria have been developed to prevent toxic effects on aquatic biota based on laboratory and field studies of metal toxicity (USEPA 1980a-e, Canada 1980). Mean pool and leachate concentrations of lead, cadmium, zinc and copper exceeded water quality criteria for chronic metal exposures, and mean leachate concentrations of cadmium, zinc and copper also exceeded criteria for maximum allowable short-term concentrations.

The importance of water metal concentrations to leachate toxicity was supported by their strong correlation with invertebrate survival and growth in leachate exposures. Cadmium and zinc concentrations in pool and leachate water, respectively, showed the strongest correlations with all toxic effects observed in invertebrates during leachate exposure. Lead, manganese and nickel concentrations also

Table 19 United States and Canada water quality criteria for heavy metals All concentrations expressed as µg/L

	Metal				
	Pb	Cd	Zn	Ni	Cu
U S EPA*					
24-hr average	3 8	0 025	47	96	5 6
Instantaneous	170	3 0	320	1800	22
Canada**	5-30	0 2	50	25-250	2 0

* Maximum allowable 24-hour average and instantaneous total recoverable metal concentrations, respectively, based on hardness of 100 mg/L as $CaCO_3$ (USEPA 1980a-e)

** Canada Department of Environment (1980) showed strong negative correlations, and each of these metals was significantly correlated with at least one invertebrate effect Copper concentrations were not strongly correlated with any invertebrate effects

The nature of the leachate and pool metal data limit the attribution of invertebrate effects to a specific metal or metal mixture The usefulness of the water metal data is reduced by the long sampling intervals (approximately monthly) and the small number of water analyses for each treatment The data allow comparison of pool and leachate composition over the entire exposure period, but do not indicate shorter-term fluctuations or trends which may have had important effects on leachate toxicity Inter-correlation of metal concentrations in pool water among treatments also limits the evaluation of individual metal toxicity (Table 20) Mean lead, cadmium and zinc concentrations were significantly inter-correlated and these metals were all strongly ($p \leq 0.10$) correlated with manganese Nickel was significantly correlated only with cadmium, and copper did not show significant correlation with any other metal in pool water

Interactions among metals in the tailings leachates may have affected the contribution of individual metals to the overall toxicity of the leachates Antagonistic interactions have been reported from studies of fish exposed to metal mixtures such as cadmium and zinc (Eaton 1973, Spehar et al 1978) and lead and copper (Ozoh 1979) However, metal interactions are often complex, as in a study

Table 20 Pearson product-moment correlation among mean pool water metal concentrations (N=6 One asterisk indicates $p \leq 0$ 10, two asterisks indicates $p \leq 0$ 05

	Cd	Zn	Mn	Ni	Cu
Pb	0 82**	0 98**	0 99**	0 56	0 15
Cd	---	0 91**	0 79*	0 86**	0 21
Zn		---	0 96**	0 66	0 13
Mn			---	0 57	0 23
Ni				---	0 47

of metal toxicity to phytoplankton (Pietilainen 1976) which found lead and cadmium acted synergistically or antagonistically when cadmium or lead, respectively, was at greater concentration Nevertheless, many studies of mixtures of three or more metals have found additive or synergistic increases in toxicity, despite the presence of reportedly antagonistic metal pairs (Brown and Dalton 1970, Eaton 1973, Hale 1977, Borgmann 1980)

Although invertebrate metal bioaccumulation has advantages over water metal concentrations as an indicator of heavy metal pollution (Nehring 1976, Nehring et al 1979), metal bioaccumulation did not closely reflect metal toxicity in this study Metal bioaccumulation was negatively correlated with invertebrate survivorship (both species) and growth (midges), but correlations were generally weaker than those with pool or leachate metal concentrations Cadmium bioaccumulation was less strongly correlated with invertebrate effects than was lead bioaccumulation, despite the stronger correlation of effects with water cadmium concentrations

The relationship between bioaccumulation and toxicity can be affected by adaptations of exposed organisms and the chemical behavior of metals Metal binding proteins which reduce the toxicity of absorbed metals have been reported in both fish and invertebrates (Bryan 1967, Dixon and Sprague 1981, Talbot and Magee 1978) Metals may also be accumulated to high concentrations without apparent toxicity in storage organs as part of normal regulation of essential metals (Bryan 1967) or as an adaptation to metal-contaminated environments (Brown 1977, 1978, The hepatopancreas of crustaceans is a major storage site for absorbed metals and may be especially important for accumulation of metals absorbed from stomach contents (Bryan 1967, Brown 1977, Wright 1980) Differences in the chemical characteristics of metals may also affect the toxicity of accumulated metals Binding to complexing agents can change the toxicity of metals without affecting bioavailability (Winner 1984) A similar effect may have occurred in leachate exposures, as cadmium rapidly decreased in the liquid phase (Harwood 1984), but remained available for uptake by crayfish, apparently from ingested solids If toxicity of metals absorbed from gut contents was reduced relative to dissolved metals, gut metal uptake may have masked the toxic significance of metal body burdens during leachate exposures

The distribution of metals among ionic, complexed and solid forms, and corresponding differences in toxicity, could have been affected by differences in the physicochemical characteristics of leachates among tailings cover treatments Minor differences in pH and hardness probably would not have significant effects on metal toxicity, although lower pH would increase the proportion of highly toxic free metal ion present (Forstner and Wittman 1980, Harwood 1984) Reduction in free metal ion concentrations would result from both complexation with inorganic and organic ligands and adsorption or precipitation to the solid phase Harwood (1984) reported reduced proportions of dialyzable metals (soluble forms, including metal ions, dissolved compounds and low-molecular weight complexes) in pool waters compared to leachates Dialysis studies indicated that metals were associated with high-molecular weight fractions in pool waters Similarly, computer modelling predicted precipitation of cadmium and zinc after equilibration in leachate pools, but indicated that lead, zinc and cadmium would occur largely as soluble complexes in the presence of humic acids (Harwood 1984)

Although complexation with organic ligands has been reported to reduce metal toxicity (Sprague 1968, Brown et al 1974), some types of dissolved organic compounds have been shown to increase the toxicity of complexed metals (Giesy et al 1977, Winner 1984) Characteristics of dissolved organic compounds such as molecular weight and chemical structure apparently affect the stability of metal organic complexes, thus affecting metal toxicity by determining the lability or availability of complexed metal ions (Bryan 1971, Giesy et al 1977) Measurable proportions of non-labile (strongly-bound) lead, cadmium and nickel occurred in most filtered leachate and pool samples from the leaf treatment and in occasional samples from all other treatments except the uncovered tailings The sludge treatment leachate showed detectable proportions of non-labile lead, cadmium and zinc in some samples, but characterization of metal species in this treatment was inconclusive (Harwood 1984) Differences in characteristics of metal organic complexes between the leaf and sludge leachate may have influenced the observed disparity in leachate toxicity despite high DOC concentrations in both pools

Implications for Exposed Populations

The type and severity of effects observed during leachate exposures have different implications for populations of crayfish and midges under similar exposure conditions Compensatory growth increases of crayfish in low-survival treatments resulted in increased biomass production in pool exposures However, this result is an artifact of food limitation, and is unlikely to occur in natural systems, where the two- to four-fold reduction in survivorship would probably have a detrimental effect on crayfish production Leachate effects would probably be more severe during full life-cycle exposure, since metal toxicity is reportedly greatest for newly-hatched crayfish (Hubschmann 1966) Reduced young-of-the-year survivorship could lead to eventual elimination of populations from habitats receiving leachate inputs, even in the absence of effects on adult crayfish

Results of midge exposures indicate that leachate toxicity could seriously affect midge populations Reductions in both growth and survivorship would markedly reduce midge biomass production under leachate exposure Inhibition of normal development would also limit the ability of a population to compensate for high mortality with increased reproduction However, midges are a highly mobile and opportunistic group with multivoltine life cycles, and populations in impacted habitats might be maintained by immigration of ovipositing females

Leachate and pool metal concentrations causing toxic effects on invertebrates in this study are comparable to those reported from contaminated sites in the Big River and its tributaries Leachate concentrations of lead, cadmium and zinc were lower than those reported in seepage from the Elvins tailings pile (Kramer 1976), but similar to samples from a drain at the Desloge pile (Harwood 1984) Concentrations of these metals in pool water were lower than concentrations reported from the Big River at Desloge (Schmitt and Finger 1982) or from lower Flat River Creek (Kramer 1976) However, metal toxicity is probably reduced in these habitats due to the antagonistic effects of water hardness (approximately twice as high in the Big River and Flat River Creek as in the leachate pools, Pyck 1974, Schmitt and Finger 1982) Leachate impacts in these streams are also reduced by dilution and loss of metals from solution, which rapidly reduce filtrable metal concentrations away from seepage inputs (Kramer 1976) Nevertheless, mobilization of metals in leachates from tailings piles and similar processes occurring in tailings-contaminated stream sediments combine to maintain elevated concentrations of dissolved metals in contaminated reaches of the Big River drainage which may have significant adverse effects on aquatic organisms

This study also indicated that inputs of organic matter or efforts to establish a vegetative cover could significantly increase the toxicity of leachates from tailings deposits. Vegetative cover caused only moderate increases in leachate toxicity relative to leachates from uncovered tailings, and data presented by Harwood (1984) suggest that vegetation impacts may be of short duration, especially compared to the long-term benefits of revegetation. However, some organic cover materials had more severe effects, reinforcing previous concerns (Novak and Hasselwander 1980) about the continuing operation of the sanitary landfill on the Desloge tailings pile. Such large-scale inputs of decomposing organic matter may substantially increase the toxicity of leachates from tailings deposits to aquatic organisms.

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